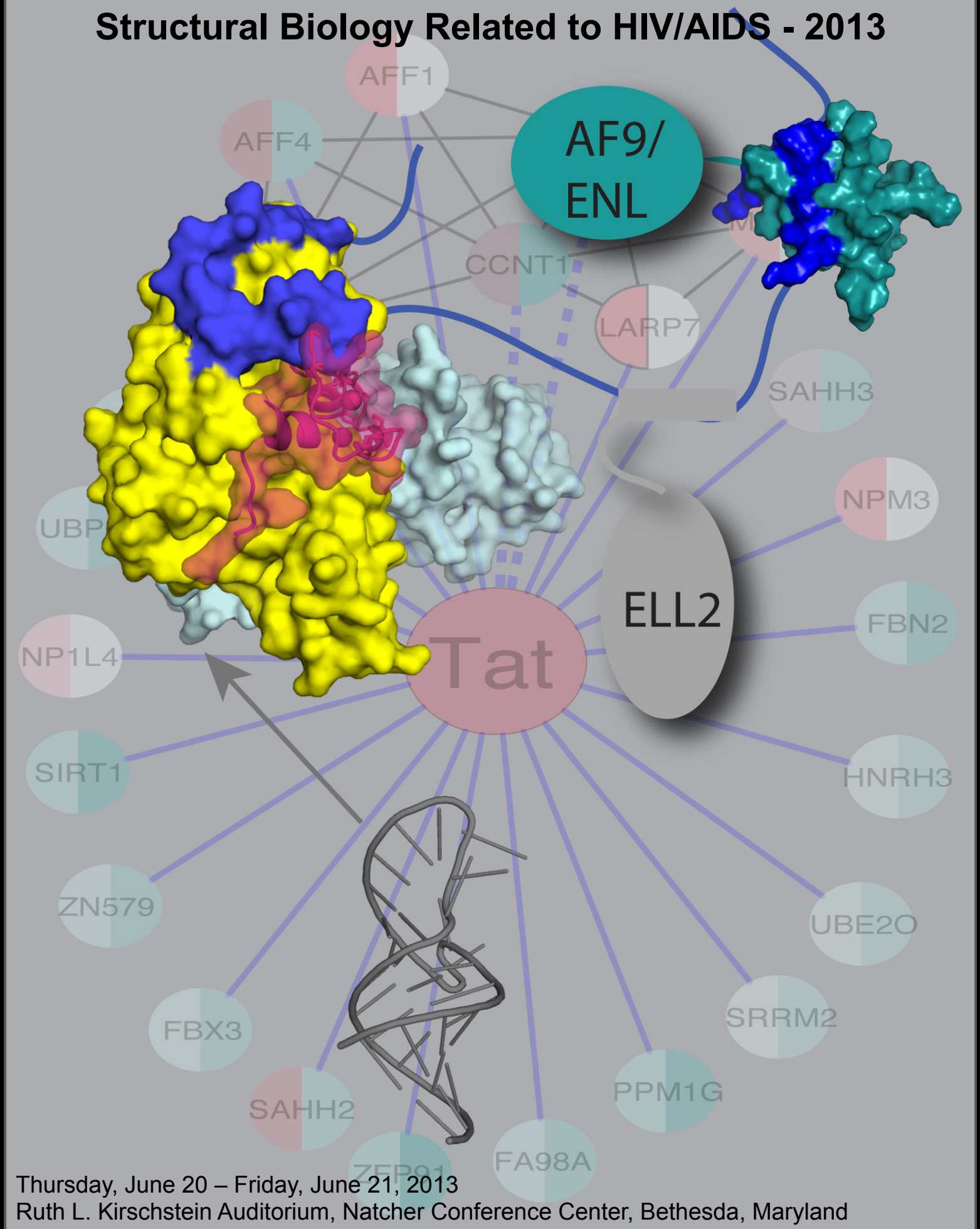
Structural Biology Related to HIV/AIDS - 2013



Thursday, June 20 – Friday, June 21, 2013
Ruth L. Kirschstein Auditorium, Natcher Conference Center, Bethesda, Maryland

NIH National Institute of General Medical Sciences

NIH National Institute of Allergy and Infectious Diseases

About the Cover:

Studies in the HARC Center revealed that HIV-1 Tat binds the super elongation complex (SEC), a multi-protein complex assembled on a flexible scaffold protein of the Af4 family. Tat is recruited to the promoter by binding to the nascent TAR RNA (gray; arrow indicates approximate RNA binding site). Structural studies from HARC show that AFF4 (blue) binds to Cyclin T, (light grey and yellow), and is partially ordered in this process (Schulze-Gahmen U. et al., 2013). Superposition of Tat (red) from the P-TEFb complex structure determined by others (Tahirov, T. et al., 2010), reveals that AFF4 binds adjacent to Tat. Also shown are other SEC components, including ELL2 (schematic) and a recently determined structure of the AF9 domain bound to AFF4 (Leach, B.I., et al., 2013) (blue and teal surface representation).

Table of Contents

Structural Biology Related to HIV/AIDS – 2013

DAY 1

8:00 – 8:05 **Michael Sakalian** (National Institute of General Medical Sciences)
Welcome/Opening Remarks

Session I: Reports from Specialized Centers (P50); Session Chair: Arthur Olson

8:05 – 9:15 The Pittsburgh Center for HIV Protein Interactions (PCHPI)
Angela Gronenborn (University of Pittsburgh)
Progress Update from the Pittsburgh Center for HIV Protein Interactions

Peijun Zhang (University of Pittsburgh)
Structure of the Mature HIV-1 Capsid by Cryo-EM and All-Atom Molecular Dynamics Simulation

Peter Cherepanov (London Research Institute)
Crystal Structures of Transportin 3 (TNPO3) in Unliganded, RanGTP- and Cargo-Bound Forms

Yong Xiong (Yale University)
Structural basis of dGTP induced allosteric activation of SAMHD1

9:15 – 10:25 The Center for HIV RNA Studies (CRNA)
Alice Telesnitsky (University of Michigan)
CRNA Overview

Victoria D'Souza (Harvard University)
Structural Mechanism of Tat binding to 7SK snRNA

Blanton Tolbert (Case Western Reserve University)
Structural studies of host-vRNA Interactions Involved in HIV Genome Splicing

Hashim Al-Hashimi (University of Michigan)
Computational and Experimental Approaches for Targeting Transient State Structures of HIV-1 RNA

10:25 – 11:00 BREAK

11:00 – 12:10 Center for the Structural Biology of Cellular Host Elements in Egress, Trafficking, and Assembly of HIV (CHEETAH)
Wes Sundquist (University of Utah)
A Role for Angiomotin in HIV-1 Budding

Adam Frost (University of Utah)
Structural Studies of ESCRT-III Assemblies

12:10 – 1:00 LUNCH

1:00 – 3:00 POSTER SESSION

Session II: Reports from Specialized Centers (P50) continued; Session Chair: Alice Telesnitsky

3:00 – 4:10 Center for HIV Accessory and Regulatory Complexes (HARC)
Nevan Krogan (University of California, San Francisco)
HARC Center: HIV Accessory and Regulatory Complexes; Overview

John Gross (University of California, San Francisco)
Assembly and Molecular Architecture of the Vif E3 Ubiquitin Ligase

Tom Alber (University of California, Berkeley)
HIV Tat Recognition of Human Transcription Elongation Factors

4:10 – 5:20 HIV Interaction and Viral Evolution Center (HIVE)
Arthur Olson (The Scripps Research Institute, La Jolla)
HIVE Overview

Eddy Arnold (Rutgers University)
Comparative Experiences in Crystallographic Fragment Screening with HIV-1 Reverse Transcriptase and Integrase

Alan Engelman (Dana-Farber Cancer Institute)
Allosteric Inhibitors Deregulate Integrase Multimerization During HIV-1 Particle Maturation

Alan Rein (NCI HIV Drug Resistance Program)
HIV-1 Gag Protein: The Role of Oligomerization in Virus Assembly

5:20 ADJOURN FOR DAY

DAY TWO

Session III: Special Presentation, Session Chair: Michael Sakalian

8:00 – 8:30 **Michael Rossmann** (Purdue University)
A Personal History of Structural Virology

Session IV: Env, Session Chair: Robert Stroud

8:30 – 9:15 P01: Structure-Based Antagonism of HIV-1 Envelope Function in Cell Entry
Irwin Chaiken (Drexel University)
Introduction to the Program on Structure-Based Antagonism of HIV-1 Envelope Function in Cell Entry

Youdong Mao (Dana Farber Cancer Institute)
Molecular Architecture of the Uncleaved HIV-1 Envelope Glycoprotein Trimer

Walther Mothes (Yale University)
Temporal Definition of HIV-1 gp120 Structural Motions in Functional Envelope Spikes

9:15 – 9:45 **Sriram Subramaniam** (National Cancer Institute)
HIV envelope glycoprotein structure and mechanisms of viral entry

9:45 – 10:15 **Peter Kwong** (NIAID Vaccine Center)
Antibody-Stabilized Env Structures

10:15 – 10:45 **Ian Wilson** (The Scripps Research Institute)
An Integrative Approach to Characterizing Broadly Neutralizing Antibodies to the HIV-1 Glycan Shield

10:45 – 11:15 BREAK

Session V: PR, Session Chair: Angela Gronenborn

11:15 – 12:00 Targeting Ensembles of Drug-Resistant HIV-Protease
Celia Schiffer (University of Massachusetts Medical School)
The Interdependency of Drug Resistance Evolution and Drug Design: HIV-1 Protease a Case Study

Rieko Ishima (University of Pittsburgh)
The Impact of Alterations in Enzyme Dynamics in Drug Resistance

Ronald Swanstrom (UNC Chapel Hill, School of Medicine)
Tracking Evolution of Drug Resistance for Highly Potent HIV Protease Inhibitors

Konstantin Zeldovich (University of Massachusetts Medical School)
Temporal tracking of acquisition of resistance and integration of complex datasets

12:00 – 1:00 LUNCH
(SAB Lunch in 2As.10; Please bring your lunch to the second floor.)

1:00 – 2:30 POSTER SESSION
(1:00 – 2:00 NIGMS Centers Scientific Review Board; Room 2As.10; second floor)

Session VI: Gag and Trim5, Session Chair: Wes Sundquist

2:30 – 3:00 **John Briggs** (EMBL Heidelberg)
Cryo-Electron Tomography Studies of HIV Assembly and Maturation

3:00 – 3:30 **Eric Freed** (NCI Frederick)
Recent ProgressiIn the Development of Potent and Broadly Active HIV-1 Maturation Inhibitors

3:30 – 4:00 **Dmitri Ivanov** (University of Texas HSC San Antonio)
Structure of the Rhesus Monkey Trim5alpha Spry Domain and Its Interactions with the HIV Capsid

Session VII: APOBEC, Session Chair: Irwin Chaiken

4:00 – 4:45 Critical Interactions of APOBEC3s: Molecular Approaches to Novel HIV Therapies
Reuben Harris (University of Minnesota)
Program Overview

Joachim Mueller (University of Minnesota)
APOBEC3 Multimerization Correlates with HIV-1 Packaging and Restriction Activity in Living Cells

Yuri Lyubchenko (University of Nebraska)
APOBEC3G Oligomerization State and Dynamics Assessed by Atomic Force Microscopy

Celia Schiffer (University of Massachusetts Medical School)
Crystal Structure of the Catalytic Domain of APOBEC3F Reveals a Novel Interaction Surface with Implications for Inhibitor Design and HIV-1 Vif Binding

4:45 ADJOURN MEETING

Mark Your Calendars!

Structural Biology Related to HIV/AIDS – 2014

Thursday June 19 – Friday June 20, 2014

Natcher Conference Center, Bethesda, Maryland

Poster Sessions

Thursday	**1:00 – 3:00**
Friday	**1:00 – 2:30**

Poster presentations are NOT assigned to either day. We have provided a sufficient number of boards for all posters to be up for both Thursday and Friday.

Posters

1. Dynamic Analysis of HIV-1 Gp120 Glycoprotein and Its Implications to Trimer Model Design

Tuoling Qiu[1] and David F. Green[1,2]
[1]Chemistry Department, Stony Brook University, Stony Brook, NY 11794; [2]Department of Applied Mathematics and Statistics, Stony Brook University, Stony Brook, NY 11794

2. Functional Characterization and Oligomeric State of Real Like Full Length gp41 Ectodomain

Koyeli Banerjee, David P. Weliky
Department of Chemistry, Michigan State University, East Lansing, MI 48824

3. Interactions of Peptide Triazole Thiols with Env gp120 Induce Irreversible Breakdown and Inactivation of HIV-1 Virions

Arangassery Rosemary Bastian[1,2], Mark Contarino[1], Kantharaju Kamanna[1], Diogo Rodrigo deMagalhaes Moreira [1,3], Kevin Freedman[4], Karyn McFadden[5], Caitlin Duffy[1], Ali Emileh[6], Jeffrey Jacobson[7], James A. Hoxie[8], Irwin Chaiken[1]
Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA, 19102[1], Department of Pharmaceutical Sciences, Federal University of Pernambuco, Reficè-PE, Brazil,[3] Department of Mechanical Engineering and Mechanics, Drexel University, Philadelphia, PA, 19104,[4] Department of Molecular Genetics and Microbiology, Duke University, Durham, N.C., 27710,[5] Department of Chemical and Biological Engineering, Drexel University, Philadelphia, PA, 19104,[6] Department of Medicine, Drexel University College of Medicine, Philadelphia, PA, 19102,[7] Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, 19104.[8]

4. Effects of the Env Membrane-Protein Interface in the Lytic Inactivation of HIV-1 by Peptide Triazole Thiols

Ramalingam Venkat Kalyana Sundaram[1,2], Cameron Abrams[3], and Irwin Chaiken[2]
[1]School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA 19104; [2]Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA 19102; [3]Chemical and Biological Engineering, Drexel University, Philadelphia, PA 19104

5. Membrane Structure Correlates to Function of LLP2 on the Cytoplasmic Tail of HIV-1 gp41 Protein

Alexander L. Boscia[1], Kiyotaka Akabori[1], Zachary Benamram[1], Jonathan A. Michel[1], Michael S. Jablin[1], Jonathan D. Steckbeck[2,3], Ronald C. Montelaro[2,3], John F. Nagle[1] and Stephanie Tristram-Nagle[1]
[1]Biological Physics Group, Physics Department, Carnegie Mellon University, Pittsburgh, Pennsylvania, USA; [2]Center for Vaccine Research and [3]Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

6. Towards Understanding the Molecular Determinants of Peptide Triazole Interactions with HIV-1 Env gp120

Rachna Aneja[1], Andrew Holmes[1], Emily Arturo[1], Caitlin Duffy[1], Huiyuan Li[1], Ali Emileh[1,3], Judith Lalonde[2], Cameron Abrams[3], Irwin Chaiken[1]

Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA 19102; [2]Chemistry, Bryn Mawr College, Bryn Mawr, PA 19010; [3]Chemical and Biological Engineering, Drexel University, PA 19104

7. Mode of Action of the Sulfhydryl Group in Virolytic Peptide Triazole Thiol Inhibitors of HIV-1 Env

Lauren D. Bailey, Caitlin Duffy, Rachna Aneja, Rosemary Bastian, Huiyuan Li, Ali Emileh and Irwin Chaiken

Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA, 19102.

8. Multiple Receptor Protocol for DOCK to Target gp41

Brian C. Fochtman[1] and Robert C. Rizzo[2,3,4]

[1]Department of Biochemistry and Cellular Biology, [2]Department of Applied Mathematics and Statistics, [3]Institute of Chemical Biology & Drug Discovery, [4]Laufer Center for Physical and Quantitative Biology Stony Brook University, Stony Brook, NY 11794

9. Computational Protein Design of Carbohydrate Binding Proteins

Yiwei Cao[1,2] and David F. Green[1,2]

[1]Department of Applied Mathematics and Statistics, Stony Brook University, Stony Brook, NY, 11794-3600; [2]Laufer Center for Physical and Quantitative Biology, Stony Brook University, Stony Brook, NY

10. Discovery of CXCR4/CCR2 Dual-Targeted Fusion Inhibitors by Structure-Based Drug Design

Jie Xia[1, 2], Hongwei Jin[1], Zhenming Liu[1], Liangren Zhang[1]*, Xiang Simon Wang[2]*

[1]State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100191; [2] Molecular Modeling and Drug Discovery Core for District of Columbia Developmental Center for AIDS Research (DC D-CFAR); Laboratory of Cheminfomatics and Drug Design, Department of Pharmaceutical Sciences, College of Pharmacy, Howard University, Washington, DC 20059

11. Use of Molecular Mimicry in the Design of HIV Fusion Inhibitors

William J. Allen[1] and Robert C. Rizzo[1,2,3]

[1]Department of Applied Mathematics and Statistics, [2]Institute of Chemical Biology & Drug Discovery, [3]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, New York, 11794

12. gp41 Ectodomain Constructs for Small Molecule-Protein Complexes

Joseph D. Walsh[1,2], Shidong Chu[1], Vladimir Sofiyev[1], Guangyan Zhou[1], Hardeep Kaur[1], Miriam Gochin[1,2]

[1]Department of Basic Science, Touro University. 1310 Club Dr., Vallejo CA 94594; [2]Department of Pharmaceutical Chemistry, UCSF, San Francisco CA 94158.

13. Targeting HIV gp41 Fusion: Binding Affinity Characterization using Thermodynamic Integration and Pharmacophore-based Scoring in DOCK6

Lingling Jiang[1] and Robert C. Rizzo[1,2,3]
[1]Department of Applied Mathematics & Statistics, [2]Institute of Chemical Biology & Drug Discovery, [3]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, NY 11794

14. Fragment Screening Targeting HIV-1 Fusion Glycoprotein-41

Shidong Chu, Miriam Gochin
Touro University California, Vallejo, CA 94592

15. Property-Based Volume Overlap as a DOCK Scoring Function

Yuchen Zhou[1] and Robert C. Rizzo[1,2,3]
[1]Department of Applied Mathematics and Statistics, [2]Institute of Chemical Biology & Drug Discovery, [3]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, NY 11794

16. Structural and Functional Characterization of Viral Fusion Peptides by Lipid Mixing Assays and Solid-State NMR

Li Xie, Ujjayini Ghosh, David Weliky
Department of Chemistry, Michigan State University, East Lansing, MI 48824

17. Structure of Cyclophilin A in Complex with HIV-1 Capsid Assembly by CryoEM

Chuang Liu and Peijun Zhang
Department of Structural Biology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15260, USA

18. Evidence for Biphasic Uncoating During HIV-1 Infection From a Novel Imaging Assay

Hongzhan Xu[1], Tamera Franks[1], Gregory Gibson[2], Kelly Huber[1], Christopher Aiken[3], Simon Watkins[2], Nicolas Sluis-Cremer[1], and Zandrea Ambrose[1]
[1]Division of Infectious Diseases, Department of Medicine and [2]Department of Cell Biology and Molecular Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA; [3]Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

19. Tetramerization of SAMHD1 Is Required for Biological Activity and Inhibition of HIV Infection

Junpeng Yan[1], Sarabpreet Kaur[1], Maria DeLucia[2,3], Caili Hao[1], Jennifer Mehrens[2,3], Chuanping Wang[1], Marcin Golczak[4], Krzysztof Palczewski[4], Angela M. Gronenborn[2,3], Jinwoo Ahn[1,2,3,*], and Jacek Skowronski[1,2,3,*]
[1]Departments of Molecular Biology and Microbiology and [4]Pharmacology, Case Western Reserve School of Medicine, Cleveland, Ohio 44106; [2]Department of Structural Biology and [3]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15260

20. HIV-2 and SIV_{mac} Accessory Virulence Factor Vpx Down-Regulates SAMHD1 Catalysis Independent of Proteasome-Dependent Degradation

Maria DeLucia[1,2,*], Jennifer Mehrens[1,2,*], Ying Wu[1,2], and Jinwoo Ahn[1,2]
[1]Department of Structural Biology and [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260; [*]These authors contributed equally.

21. A Single Amino Acid in the Sterile Alpha Motif (SAM) Domain of Human SAMHD1 Alters Susceptibility to a Subset of Simian Immunodeficiency Virus (SIV) Virulence Factors Vpr and Vpx

Ying Wu[1,2], Jennifer Mehrens[1,2], Maria DeLucia[1,2], and Jinwoo Ahn[1,2]
[1]Department of Structural Biology and [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260

22. Analysis of the Conformational Dynamics of Viral Proteins by HDX

Devrishi Goswami[1], Samantha Yost[2], Steve Tuske[3], Edward Arnold[3], Joseph Marcotrigiano[2] and Patrick Griffin[1]
[1]The Scripps Research Institute; [2]Rutgers University - Center for Advanced Biotechnology and Medicine; [3]Rutgers University, Chemistry & Chemical Biology

23. Small Angle X-Ray Scattering Analysis of the HIV-1 vRNA 5´ UTR Initiation Complex Reveals a tRNA-Like Fold

Christopher P. Jones, William Cantara, Erik Olson, and Karin Musier-Forsyth
Department of Chemistry and Biochemistry, Center for RNA Biology, and Center for Retroviral Research, Ohio State University, Columbus, OH 43210

24. Small Angle X-ray Scattering Studies of the HIV-1 Reverse Transcriptase Initiation Complex

William C. Ho[1], Steve Tuske[1], Matthew T. Miller[1], Joseph D. Bauman[1], Jennifer T. Miller[2], Stuart F.J. Le Grice[2], and Eddy Arnold[1]
[1]Center for Advanced Biotechnology and Medicine, Department of Chemistry and Biology, Rutgers University, Piscataway, New Jersey, USA. [2]HIV Drug Resistance Program, NCI-Frederick Cancer Research and Development Center, Frederick, Maryland, USA

25. 5,6-Dihydro-5-aza-2'-deoxycytidine Potentiates the Anti-HIV-1 Activity of Ribonucleotide Reductase Inhibitors

Jonathan M. Rawson, Richard H. Heineman, Lauren B. Beach, Jessica L. Martin, Erica K. Schnettler, Michael J. Dapp, Steven E. Patterson, and Louis M. Mansky
Institute for Molecular Virology, University of Minnesota, Minneapolis, MN 55455, USA

26. APOBEC3G Monomers and Oligomers Bind Single-Stranded DNA

Ming Li and Reuben S. Harris
Department of Biochemistry, Molecular Biology & Biophysics, Institute for Molecular Virology, Center for Genome Engineering, University of Minnesota, Minneapolis, Minnesota, USA 55455

27. Novel APOBEC3G Properties Allow it to be an Efficient Mutator of Newly Synthesized HIV-1 DNA

Roni Nowarski[1, 2], Edan Kenig[1], Prabhu Ponnandy[1] and Moshe Kotler[1]
[1]Department of Pathology and the Lautenberg Center for General and Tumor Immunology, the Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel; [2]Present address: Department of Immunobiology, Yale University School of Medicine, New Haven, CT 06520, USA

28. Mouse APOBEC3 Deaminase: Biochemical Properties and Possible Regulation by Phosphorylation

Smita Nair, Silvia Sanchez-Martinez and Alan Rein
Retrovirus Assembly Section, HIV Drug Resistance Program, National Cancer Institute, Frederick, MD-21702.

29. Time-Resolved NMR of DNA Cytosine Deamination by APOBEC3G

Stefan Harjes, William Solomon, Ming Li, Kuan-Ming Chen, Elena Harjes, Reuben S. Harris, and Hiroshi Matsuo
Department of Biochemistry, Molecular Biology, and Biophysics, Institute for Molecular Virology, University of Minnesota, Minneapolis, Minnesota, USA

30. NMR Structure of Human Restriction Factor APOBEC3A: Substrate Binding and Enzyme Specificity

In-Ja L Byeon[1,2], Jinwoo Ahn[1,2], Mithun Mitra[3], Chang-Hyeock Byeon[1,2], Kamil Hercík[3] , Jozef Hritz[1], Lisa M Charlton[1,2], Judith G Levin[3] & Angela M Gronenborn[1,2]
[1]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA 15261, USA; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA; [3]Section on Viral Gene Regulation, Program on Genomics of Differentiation, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892-2780, USA

31. Small Molecule Inhibitors of APOBEC3G DNA Cytosine Deaminase

Daniel A. Harki[†*], Margaret E. Olson[†], Angela L. Perkins[†], Daniel Abate Pella[†], Ming Li[‡], Anurag Rathore[‡], Michael A. Carpenter[‡], and Reuben S. Harris[‡]
[†]Department of Medicinal Chemistry, University of Minnesota, 717 Delaware Street S.E., Minneapolis, MN 55414, United States; [‡]Department of Biochemistry, Molecular Biology & Biophysics, University of Minnesota, 321 Church Street S.E., Minneapolis, MN 55455, United States

32. Cryo-Electron Tomography of Cryo-FIB Sectioned Mammalian Cells

Ke Wang[1,3], Korrinn Strunk[2,3], Gongpu Zhao[1], Yun Han[1], Jennifer L. Gray[2] and Peijun Zhang[1,2]
[1]Department of Structural Biology, School of Medicine; [2]Department of Mechanical Engineering and Materials Science, School of Engineering, University of Pittsburgh, Pittsburgh, PA 15260, USA

33. A Small Molecule Inhibitor Targeting Capsid and Nuclear Import Blocks HIV-1 Replication

Atsuko Hachiya[1,2], Christie Pautler[1], Jennifer Moran[1], Sanath Janaka[1], Karen A. Kirby[1], Eleftherios Michailidis[1], Yee T. Ong[1], Shinichi Oka[3], Michael A. Parniak[4], Wataru Sugiura[2], KyeongEun Lee[5], Vineet N. KewalRamani[5], Kamalendra Singh[1], and Stefan G. Sarafianos[1,6]

[1]University of Missouri, Department of Molecular Microbiology & Immunology, Columbia, MO, 65211; [2]Clinical Research Center, National Hospital Organization Nagoya Medical Center, Department of Infectious Diseases and Immunology, Nagoya, 4600001, Japan; [3]AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, 1628655, Japan; [4]University of Pittsburgh, Department of Molecular Genetics & Biochemistry, Pittsburgh, PA, 15219; [5]National Cancer Institute, HIV Drug Resistance Program, Frederick, MD, 21702; [6]University of Missouri, Department of Biochemistry, Columbia, MO, 65211

34. A Detailed Analysis of the Role of TNPO3 in HIV-1 Infection

Ilker Oztop[1], KyeongEun Lee[2], Goedele N Maertens[3], Peter Cherepanov[4], Vineet N KewalRamani[2], Alan Engelman[1]

[1]Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, MA, 02215; [2]HIV Drug Resistance Program, National Cancer Institute, Frederick, MD, 21702; [3]Department of Medicine, Imperial College London, UK; [4]Clare Hall Laboratories, London Research Institute, Cancer Research UK.

35. Modeling the HIV-1 Intasome

Barry C. Johnson[1], Mathieu Métifiot[2], Yves Pommier[2], and Stephen H. Hughes[1]

[1]HIV Drug Resistance Program, Frederick National Laboratory, Frederick, MD 21702, USA and [2]Laboratory of Molecular Pharmacology, National Cancer Institute, Bethesda, MD 20892, USA

36. Apo-Architecture and Assembly of a DNA Invasion Machine: HIV Integrase and Its Inhibition

Ravi S.Bojja, Mark D. Andrake, George Merkel, Steven Weigand, Roland L. Dunbrack, Jr., and Anna Marie Skalka

Fox Chase Cancer Center, Philadelphia, PA

37. The A128T Resistance Mutation Reveals Aberrant Protein Multimerization as the Primary Mechanism of Action of Allosteric HIV-1 Integrase Inhibitors

Lei Feng[1], Nivedita Jena[2], Amit Sharma[1], Alison Slaughter[1], Jacques J. Kessl[1], Alan Engelman[3], James R Fuchs[2], Mamuka Kvaratskhelia[1]

[1]The Ohio State University, Center for Retrovirus Research, College of Pharmacy , Columbus, OH, 43210, [2]The Ohio State University, Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, Columbus, OH, 43210, [3]Dana-Farber Cancer Institute, Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, & Department of Medicine, Harvard Medical School, Boston, MA, 02115

38. Hydrogen Bond Interactions of Inhibitory Molecules with Amino Acids of HIV-1 Integrase within a 4.0Å Radius of the Active Site: Inhibitors Classified as Drug-like and Nondrug-like According to Lipinski's "Rule of Five"

Julie B. Ealy, Sarah Israel, Talia Katz, Robert Huff, and Paolo Flauta

Penn State University Lehigh Valley, Center Valley, PA

39. Allosteric Integrase Inhibitor Potency is Determined Through the Inhibition of HIV-1 Particle Maturation

Kellie A Jurado[1], Hao Wang[1], Lei Fang[2], Jacques J Kessl[2], Yasuhiro Koh[1], Weifeng Wang[1], Allison Ballandras-Colas[1], Pratiq A Patel[3], James R Fuchs[3], Mamuka Kvaratskhelia[2], Alan Engelman[1]

[1]Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, MA 02215; [2]Center for Retrovirus Research and Comprehensive Cancer Center, College of Pharmacy and [3]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210

40. Novel Approach to Developing HDAC Benchmarking Sets – An Aid to SBDD of Isoform-Selective HDAC Inhibitors for Disrupting HIV Latency

Terry-Elinor Reid[1,2],Jie Xia[1,2,3],Napoleon Manian[2,4], Liangren Zhang[3], Xiang Simon Wang[1,2]

[1]Molecular Modeling and Drug Discovery Core for District of Columbia Developmental Center for AIDS Research (DC D-CFAR); [4]Center of Excellence College of Pharmacy; Laboratory of Cheminfomatics and Drug Design, [2]Department of Pharmaceutical Sciences, College of Pharmacy, Howard University, Washington, District of Columbia 20059; *[3]State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100191*

41. Development of Isoform-Specific Inhibitors of HDACS to Probe Expression of Latent HIV-1 Genomes

Garland R Marshall[1], Sergio Valente[3], Rino Ragno[3], Antonello Mai[3], Lee Ratner[2]

[1]Washington University, Biochemistry & Molecular Biophysics, St. Louis, MO, 63110, [2]Washington University, Medicine/Molecular Oncology, St. Louis, MO, 63110, [3]Sapienza Universita di Roma, Dipartimento di Chimica e Tecnologie del Farmaco, Rome, 00185, Italy

42. The AFF4 Scaffold Binds Human P-TEFb Adjacent to HIV Tat

Ursula Schulze-Gahmen[1], Heather Upton[1], Andrew Birnberg[1], Nevan J. Krogan[2,3,4], Qiang Zhou[1], Tom Alber[1,5]

[1]Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720 USA; [2]Department of Cellular and Molecular Pharmacology, University of California, 1700 4th Street, San Francisco, CA 94158 USA; [3]California Institute for Quantitative Biosciences, QB3, San Francisco CA 94158 USA; [4]J. David Gladstone Institutes, San Francisco, CA, 94158, USA; [5]California Institute for Quantitative Biosciences, QB3, Berkeley, CA 94720 USA

43. Towards Developing a Structure Based Mechanism of Splicing Repression by hnRNP A1 at ssA7 on HIV-1

Jeffrey D. Levengood, Carrie Rollins, and Blanton Tolbert

Case Western Reserve University, Department of Chemistry, Cleveland, OH 44106

44. Structural Fidelity and NMR Relaxation Analysis in a Prototype RNA Hairpin

George M. Giambaşu,[2] Niel Henriksen,[1] Thomas E. Cheatham, III,[1] Darrin M. York[2] and David A. Case[2]

[1]Department of Medicinal Chemistry, College of Pharmacy, University of Utah, 2000 East 30 South Skaggs 105, Salt Lake City, UT 84112; [2]BioMaPS Institute for Quantitative Biology and Department of Chemistry and Chemical Biology Rutgers University 174 Frelinghuysen Rd, Piscataway, NJ 08854

45. Advances in NMRViewJ for Analysis of NMR Spectra of RNA

Shawn Barton[1,2] Xiao Heng[1,2], Michael F. Summers[1,2] & Bruce A. Johnson[2,3]
[1]Howard Hughes Medical Institute, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250; [2]Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250; [3]One Moon Scientific, Inc., 839 Grant Ave., Westfield, NJ 07090

46. SHAPE Analysis of HIV Rev Response Element (RRE) *In Vitro* and *In Vivo*

Yun Bai[1], Jennifer A. Doudna[1,2,3,4]
[1]Department of Molecular and Cell Biology, [2]Howard Hughes Medical Institute, [3]Department of Chemistry, University of California, Berkeley, California 94720, USA; [4]Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA

47. Characterization of the Interactions Between HIV Rev Protein and Host RNA Helicase DDX21 *In Vitro*.

Li Zhou and James R Williamson
Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA, 92037

48. Toward Understanding the Role of Human DEAD-Box Protein 1 (DDX1) in Rev-Dependent Export of HIV-1 RNA

J. A. Hammond, Li Zhou, David P. Millar, Jamie R. Williamson
The Scripps Research Institute Department of Molecular Biology, 10550 North Torrey Pines Road, La Jolla, California, 92037

49. Imaging DDX1 Interaction with HIV-1

Ammeret Rossouw and David Grunwald
UMass Medical School, RNA Therapeutics Institute, Worcester, Massachusetts

50. Single-Molecule Studies of HIV-1 Rev Assembly on the Rev Response Element

Rajan Lamichhane[1], John Hammond[1], Rebecca Filbrandt[2], Souad Naji[2], Larry Gerace[2], James Williamson[1] and David P. Millar[1]
[1]Department of Integrative Structural and Computational Biology, and [2]Department of Cell and Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037 USA

51. Evidence that the Rous Sarcoma Virus Gag Protein Captures its Genome in the Nucleus

Rebecca J. Kaddis[1], Estelle Chiari-Fort[1], Darrin V. Bann[1], Breanna L. Rice[1], Matthew S. Stake[1], and Leslie J. Parent[1, 2]
Penn State College of Medicine, [1]Department of Medicine, and [2]Department of Microbiology and Immunology, Hershey, PA 17033

52. Investigate the Dimerization Mechanism of the 5' Leader RNA Genome in Simian Immunodeficiency Virus

Thao Tran, Jennifer Bohn, Michael Summers Ph. D.
Howard Hughes Medical Institute and Chemistry/Biochemistry Department, University of Maryland, Baltimore County, MD

53. Structural Characterization of the 30 kDa HIV-1 RNA Dimerization Initiation Site by Cryo-EM

Rossi Irobalieva[1,2], Xiao Heng[3], Steven J. Ludtke[1,2], Michael F. Schmid[1,2], Michael F. Summers[3], Wah Chiu[1,2]

[1]Graduate Program in Structural and Computational Biology and Molecular Biophysics, Baylor College of Medicine, Houston, TX; [2]National Center for Macromolecular Imaging, Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX; [3]Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, Baltimore MD

54. Host RNA Helicase Incorporated into HIV-1 Virions: Chaperone Activity Promotes the Infectivity of Progeny Virions

Ioana Boeras[1], Amit Sharma[2] and Kathleen Boris-Lawrie[1]

[1] HIV Center for RNA, Department of Veterinary Biosciences, Center for Retrovirus Research, Center for RNA Biology, Ohio State University, Columbus Ohio, USA; [2] Center for Retrovirus Research, College of Pharmacy, Ohio State University, Columbus Ohio, USA

55. GB Virus Type C E2 Protein Interferes with HIV-1 Gag Plasma Membrane Targeting Through Inducing ADP-Ribosylation Factors 1 Degradation

Chenliang Wang[1,2], Christine L. Timmons[1], Huanliang Liu[2*], Bindong Liu[1]

[1]Center for AIDS Health Disparities Research, Meharry Medical College, Nashville, Tennessee and [2]Institute of Gastroenterology and Institute of Human Virology, Sun Yat-sen University, Guangzhou, Guangdong, Peoples of Republic of China

56. The mRNA-Binding Protein YB1 Participates in the Assembly of a Murine Betaretrovirus

Darrin V. Bann, Andrea R. Beyer, and Leslie J. Parent

Departments of Medicine and Microbiology & Immunology, Penn State College of Medicine, Hershey, PA

57. Characterization of HIV-1 Matrix Interactions

Ayna Alfadhli, Rachel Sloan, Isabel Cylinder, Sarah Gabriel, Claudia López, Henry McNett, and Eric Barklis

Vollum Institute and Department of Microbiology, Oregon Health and Science University, Portland, Oregon 97239

58. Identification of a Matrix Mutation that Globally Rescues Env Incorporation Defects: Implications for Matrix Structure and Env Recruitment

Philip R Tedbury, Sherimay Ablan, Eric O Freed

Virus-Cell Interaction Section, HIV Drug Resistance Program, NCI-Frederick, Frederick, MD.

59. Gibbon Ape Leukemia virus Envelope (GaLV Env), an unexpected Vpu target.

Marc C. Johnson, Sanath K. Janaka, Tiffany M. Lucas, Terri D. Lyddon, Devon A. Gregory

Department of Molecular Microbiology and Immunology, University of Missouri-School of Medicine, Columbia, MO 65211

60. Vpu and Host Protein Complexes

Shujun Yuan[1], Martin Lynn[1], Wu Shenping[1], Shenheng Guan[2], Yifan Cheng[1], Robert M. Stroud[1]
[1]Department of Biochemistry and Biophysics, University of California San Francisco, San Francisco, CA; [2]Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA

61. Analysis of HTLV-1 Particle Morphology and Gag Stoichiometry

Jose O. Maldonado[1,2], Iwen F. Grigsby[1,2], Sheng Cao[1,2], Wei Zhang[1,2], and Louis M. Mansky[1,2,3]
[1]Institute for Molecular Virology, [2]Department of Diagnostic and Biological Sciences, School of Dentistry, [3]Department of Microbiology, Medical School, University of Minnesota, Minneapolis, MN 55455

62. Charged Residues in Surface-Exposed Capsid Loops Mediate Immature Retroviral Assembly and Maturation

Katrina J. Heyrana, Tam-Linh N. Nguyen, Maria C. Bewley, and Rebecca C. Craven
Department of Microbiology and Immunology, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033

63. Magic Angle Spinning NMR Reveals Sequence-Dependent Structural Plasticity, Dynamics, and the Spacer Peptide 1 Conformation in HIV-1 Capsid Protein Assemblies

Yun Han[1,2], Guangjin Hou[1,2], Manman Lu[1,2], Christopher L. Suiter[1,2], Jinwoo Ahn[2,3], In-Ja L. Byeon[2,3], Andrew S. Lipton[4], Sarah Burton[4], Ivan Hung[5], Peter Gor'kov[5], Zhehong Gan[5], William Brey[5], David Rice[6], Angela M. Gronenborn[2,3], and Tatyana Polenova[1,2]
[1]Department of Chemistry and Biochemistry, University of Delaware, Newark, DE; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA; [4]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA; [5]National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL; [6]Agilent Technologies, Inc., 5301 Stevens Creek Boulevard, Santa Clara, CA

64. RNA-Mediated Regulation of p15NC Processing: Revisited

Marc Potempa[1], Sook-Kyung Lee[2], and Ronald Swanstrom[1,2]
[1]Department of Microbiology and Immunology, [2]Department of Biochemistry and Biophysics, and the UNC Center for AIDS Research, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

65. Electron Cryo-Tomography Studies of Maturing HIV-1 Viral Particles

[1]Cora L. Woodward, [1,2]Grant J. Jensen
[1]Division of Biology and [2]HHMI, California Institute of Technology, 1200 E California Blvd, Pasadena, CA 91125, USA

66. Revealing the Structure of the HIV-1 Capsid

Juan R. Perilla[1], Gongpu Zhao[2,3], Peijun Zhang[2,3], and Klaus Schulten[1]
[1]Department of Physics and Beckman Institute, University of Illinois Urbana-Champaign, 61801; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15260; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15260

67. Probing Dynamics in HIV-1 Capsid Protein Assemblies by a Hybrid Solid-State NMR / Molecular Dynamics Approach

Guangjin Hou[1,2], Huilan Zhang[1,2], Christopher L. Suiter[1,2], Yun Han[1,2], Suvrajit Maji[3], Judith Klein[3], Christopher J. Langmead[3], Jinwoo Ahn[2,4], In-Ja L. Byeon[2,4], Angela M. Gronenborn[2,4], Tatyana Polenova[1,2]

[1]Department of Chemistry and Biochemistry, University of Delaware, Newark; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, 1051 Biomedical Science Tower 3, 3501 Fifth Ave., Pittsburgh; [3]Computer Science Department, Carnegie Mellon University, Gates Hillman Center, 5000 Forbes Avenue, Pittsburgh; [4]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh

68. Structure of the Mature HIV-1 Capsid

Gongpu Zhao[1,2], Juan R. Perilla[3], Ernest L. Yufenyuy[2,4], Xin Meng[1,2], Jiying Ning[1,2], Jinwoo Ahn[1,2], Angela M. Gronenborn[1,2], Klaus Schulten[3], Christopher Aiken[2,4] and Peijun Zhang[1,2]

[1]Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA; [2]Pittsburgh Center for HIV Protein Interactions, Pittsburgh, PA 15260, USA; [3]Department of Physics and Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA; [4]Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

69. Electron Tomography of HIV-1 Infection in Gut-Associated Lymphoid Tissue

Mark S. Ladinsky[1], Collin Kieffer[1], Gregory Olson[3], Douglas S. Kwon[3] and Pamela J. Bjorkman[1,2]

[1]Division of Biology 114-96 and [2]Howard Hughes Medical Institute, California Institute of Technology, 1200 East California Boulevard, Pasadena, CA 91125; [3]Ragon Institute of MGH, MIT and Harvard, 400 Technology Square, Cambridge, MA 02139;

70. Capturing Enveloped Viruses on Affinity Grids for Downstream Cryo-Electron Tomography Applications

Gabriella Kiss[1], Xuemin Chen[1], Jens M. Holl[1], Melinda A. Brindley[1], Patricia Campbell[2], Lauren A. Byrd-Leotis[2], John Steel[2], David Steinhauer[2], Claudio L. Afonso[3], Richard K. Plemper[1], Paul Spearman[1], Deborah F. Kelly[4], and Elizabeth R. Wright[1]

[1]Division of Pediatric Infectious Diseases. Department of Pediatrics. Emory University School of Medicine. Children's Healthcare of Atlanta. Atlanta, GA 30322; [2]Department of Microbiology and Immunology. Emory University School of Medicine. GA 30322; [3]USDA, ARS, Southeast Poultry Research Laboratory, Athens, Georgia, USA; [4]Virginia Tech Carilion Research Institute, Roanoke, VA 24016

71. Optical Trapping and Sorting of the Human Immunodeficiency Viruses at Single-Molecule Level

Yuanjie Pang, Hanna Song, Jin H. Kim and Wei Cheng*

*Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, 428 Church Street, Ann Arbor, MI 48109, USA. *Correspondence: chengwe@umich.edu*

72. Deep Sequencing of Protease Inhibitor Resistant HIV Reveals Patterns of Mutations in Gag

Max W. Chang[1], Jinyun Yuan[1], Glenn Oliveira[2], Samuel Levy[2], Jason F. Okulicz[3,4], and Bruce E. Torbett[1]

[1]Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA; [2]Scripps Genomic Medicine, Scripps Translational Science Institute, La Jolla, CA; [3]Infectious Disease Clinical Research Program, Uniformed Services University of the Health Sciences, Bethesda, MD; [4]Infectious Disease Service, San Antonio Military Medical Center, San Antonio TX

73. Understanding the Function of Drug Resistance Mutations in the HIV Gag Polyprotein

Divita Garg, Sebastian Breuer, Bruce E. Torbett

The Scripps Reseach Institute, La Jolla, CA

74. Selection for Resistance to Potent New HIV-1 Protease Inhibitors and Analyzed Using Deep Sequencing

Sook-Kyung Lee[1], Shuntai Zhou[1], Celia Schiffer[2], and Ronald Swanstrom[1]

[1]Department of Biochemistry and Biophysics, and the UNC Center for AIDS Research, University of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts, Worcester, MA

75. Differential Flap Dynamics in Wild-Type and a Drug Resistant Variant of HIV-1 Protease Detected by NMR Relaxation and MD Simulations

Rieko Ishima[1], Wazo Myint[1], Yufeng Cai[2], Nese Kurt Yilmaz[2], Celia A. Schiffer[2]

[1]Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA15260; [2]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA01605.

76. Protein Stability, Drug Resistance, and Fitness Landscapes of HIV-1 Protease

Omar Haq, William Flynn, R. S. K. Vijayan, Anthony Felts, Ronald M. Levy

Department of Chemistry and Chemical Biology, Rutgers University

77. Extreme Entropy-Enthalpy Compensation Due to Cooperative Mutations in the Flap Region of HIV-1 Protease

Neşe Kurt-Yılmaz, Nancy M. King, Moses Prabu-Jeyabalan, Jennifer E. Foulkes-Murzycki, Rajintha M. Bandaranayake, Madhavi N. L. Nalam, Ellen A. Nalivaika, and Celia A. Schiffer

Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA 01605

78. Advancing the Discovery of Allosteric Inhibitors of HIV Protease Using Virtual Screens on FightAIDS@Home

Alexander L. Perryman, Ying-Chuan Lin, Subash Velaparthi, Max Chang, Michael Baksh, Stefano Forli, Srinivas Reddy Chirapu, Theresa Sample, David Goodsell, C. David Stout, Valery Fokin, M.G. Finn, Bruce Torbett, John Elder, and Arthur J. Olson

The Scripps Research Institute, La Jolla, CA, USA.

79. Using Absolute Binding Free Energy Methods to Identify True Binders to Allosteric Sites on HIV Protease

Nanjie Deng, Peng He, Alex Perryman, Stefano Forli, Emilio Gallicchio, Art Olson, Ronald Levy
Department of Chemistry, Rutgers University, Piscataway, New Jersey; The Scripps Research Institute, La Jolla, California

80. Evaluating the Role of Macrocycles in the Susceptibility of Hepatitis C Virus NS3/4A Protease Inhibitors to Drug Resistance

Akbar Ali,[1] Cihan Aydin,[1] Reinhold Gildemeister,[1] Keith P. Romano, Hong Cao,[1] Ayşegül Özen,[1] Djade Soumana,[1] Alicia Newton,[2] Christos J. Petropoulos,[2] Wei Huang,[2] and Celia A. Schiffer[1]
[1]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01605, United States; [2]Monogram Biosciences, South San Francisco, California, United States

81. Probing Molecular Interactions of Protease with Small Molecules and Gag Cleavage Junctions, and Matrix with the Lipid Bilayer

Tiefenbrunn, T[1]., Forli, S.[1], Gonzalez, A[2]., Baksh, M. M.[3], Chang, M. W.[4], Tsai, Y.-S.[2], Lin, Y.-C.[5], Perryman, A. L.[1], Rhee, J.-K.[3], Torbett, B.E.[4], Olson, A. J.[1], Soltis, M.[2], Elder, J. H.[5], Finn, M. G.[3], Stout, C. D.[1]
Departments Of [1]ICSB, [3]Chemistry, [4]MEM, [5]IMS, TSRI, 10550 N. Torrey Pines Rd., La Jolla, CA 92037; [2]SSRL, SLAC, 2575 Sand Hill Road MS 99, Menlo Park, CA 95124

Progress Update from the Pittsburgh Center for HIV Protein Interactions

Angela M. Gronenborn

Department of Structural Biology, University of Pittsburgh School of Medicine

The Pittsburgh Center for HIV-Protein Interactions (PCHPI) is focused on understanding the interaction of host proteins with HIV-1 during the so-called early stages of HIV-1 infection, those that occur after viral membrane fusion and prior to integration. During the past year, the PCHPI welcomed several new investigators (and areas of methodological expertise) into the collaborative effort to understand these events. In addition, during the past year, PCHPI members made significant progress toward understanding the capsid assembly (Zhao et al, Nature, published May 30 2013), the interaction of Trim5α with Capsid (Yang et al, 2012,PNAS 109(45):18372), APOBEC3A structure and catalysis (Byeon et al, Nature Communications, in press), the structure of TNPO3, and the biochemistry and structure of SAMHD1 as well as the interaction of SAMHD1 with various Vpx (and Vpr) proteins in complex with DCAF1-Cullin4A-RING E3 ubiquitin ligase (Yan et al 2013, J Biol Chem 288(15): 10406). Finally, the center has also continued its effort to develop solid-state NMR for analyzing assembled Capsid (Yan et al, 2013 Acc Chem Res doi: 10.1021/ar300309s; Hou et al, J Am Chem Soc. 135(4):1358-68). We will briefly summarize the major findings from our studies, highlighting structural studies of APOBEC3A, the assembled Capsid, TNPO3, and SAMHD1.

Structure of the Mature HIV-1 Capsid by Cryo-EM and All-Atom Molecular Dynamics Simulation

Gongpu Zhao[1], Juan R. Perilla[2], Ernest L. Yufenyuy[3], Xin Meng[1], Jiying Ning[1], Jinwoo Ahn[1], Angela M. Gronenborn[1], Klaus Schulten[2], Christopher Aiken[3] and Peijun Zhang[1]

[1]Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA; [2]Department of Physics and Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA; [3]Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

Retroviral capsid proteins are structurally conserved yet assemble into different morphologies. The mature Human Immunodeficiency Virus-1 (HIV-1) capsid is best described by a “fullerene cone” model, in which hexamers of the capsid protein (CA) are linked to form a hexagonal surface lattice that is closed by incorporating twelve CA pentamers. HIV-1 CA contains an N-terminal domain (NTD), comprising seven α-helices and a β-hairpin, a C-terminal domain (CTD) that dimerizes and contains four α helices with a 3_{10}-helix, and a flexible linker connecting the two structural domains. Structures of the CA assembly units have been determined by X-ray crystallography; however, structural information on the assembled capsid and on contacts between the assembly units is incomplete. Here, we report the cryo-electron microscopy (cryo-EM) structure of a tubular HIV-1 CA assembly at 8 Å resolution and the 3D structure of a native HIV-1 core by cryo-electron tomography (Cryo-ET). The structure of the tubular assembly reveals, at the three-fold interface, a three-helix bundle that exhibits critical hydrophobic interactions. Mutagenesis studies confirmed that hydrophobic residues in the center of the three-helix bundle are critical for capsid assembly and stability, and for viral infectivity. The cryo-EM structures permitted unambiguous modeling by large-scale molecular dynamic (MD) simulation, resulting in all-atom models for the hexamer-of-hexamer and pentamer-of-hexamer elements as well as for the entire capsid. Incorporation of pentamers results in stronger trimer contacts and induces acute surface curvature. The complete atomic HIV-1 capsid model provides a platform for further studies of capsid function and for targeted pharmacological intervention.

Crystal Structures of Transportin 3 (TNPO3) in Unliganded, RanGTP- and Cargo-Bound Forms

Goedele N. Maertens[1], Nicola Cook[2], Stephen A. Hare[1], Saumya S. Gupta[1], Ariberto Fassati[3] and Peter P. Cherepanov[1,2]

[1]Division of Medicine, Imperial College London, UK; [2]Clare Hall Laboratories, CRUK London Research Institute, Blanche Lane, South Mimms, UK; and [3]Wohl Virion Centre, University College London, UK.

TNPO3 (also known as Transportin-SR2) is a cellular karyopherin b-like protein involved in nuclear import of SR proteins. TNPO3 has been implicated in HIV-1 replication, although its precise role in the viral life cycle remains debated. Our proteomic analyses confirmed that TNPO3 binds a wide range of proteins involved in mRNA metabolism. The majority of TNPO3 interactors contain both RNA recognition motif (RRM) and Arg-Ser repeat (RS) domains (such as ASF/SF2 and CPSF6), although small subsets included proteins containing only an RS (such as CYP4 and CYPG) or RRM (such as RBM14) domain. Results of pull down assays with recombinant ASF/SF2 phosphorylated by an RS-specific kinase suggest that its interaction with TNPO3 primarily depends on the RS domain, although a weak interaction with the RRM-only portion of ASF/SF2 could be detected.

We have now determined crystal structures of human TNPO3 protein in its non-liganded, RanGTP- and cargo-bound forms. Similar to other members of the karyopherin b family, TNPO3 is an a-helical protein, consisting of 20 HEAT repeats. The overall shape of TNPO3 is reminiscent of a split-ring washer, and its conformation varies dramatically depending on crystal packing interactions, RanGTP or cargo binding. This profound flexibility is a common characteristic of karyopherin b -like proteins.

TNPO3 engages the phosphorylated RS domain of ASF/SF2 using HEAT repeats 15-18. Phospho-Ser and Arg residues of the RS domain are involved in a network of salt bridges with complementary Arg and Glu residues exposed on the inner concave surface of TNPO3. The RRM2 domain of ASF/SF2 is wedged between TNPO3 HEAT repeats 4-7 and 19-21. Mutations within the RS-binding region of TNPO3 severely perturb interactions with ASF/SF2 and CPSF6. Our data provide a structural basis for the cellular function of TNPO3 and a detailed structural template for ongoing virology studies.

Structural Basis of dGTP Induced Allosteric Activation of SAMHD1

Xiaoyun Ji[1*], Ying Wu[2,3*], Junpeng Yan[4*], Jennifer Mehrens[2,3], Haitao Yang[1], Maria DeLucia[2,3], Caili Hao[4], Angela Gronenborn[2,3], Jacek Skowronski[3,4], Jinwoo Ahn[2,3]†, and Yong Xiong[1]†

[1]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520, USA, [2]Department of Structural Biology and [3]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, [4]Department of Molecular Biology and Microbiology, Case Western Reserve School of Medicine, Cleveland, OH 44106.
**These authors contributed equally to this work. † To whom correspondence should be addressed.*

SAMHD1, a deoxyribonucleoside triphosphate triphosphohydrolase (dNTPase), plays a key role in human innate immunity. It inhibits infection of blood cells by retroviruses, including HIV, and prevents the development of the autoinflammatory Aicardi-Goutières syndrome (AGS). The structure of apo-SAMHD1 is a dimer[1], and in the presence of dGTP the protein assembles into catalytically active tetramers[2]. Here, we present a 1.8 Å resolution crystal structure of the tetrameric SAMHD1-dGTP complex. The structure reveals an elegant allosteric mechanism of activation via dGTP-induced tetramerization of two inactive dimers. dGTP binding to four allosteric sites enables tetramerization and further induces a conformational change in the substrate-binding pocket to yield the catalytically active enzyme. The mechanism was probed and confirmed by structure-based biochemical and cell biological assays. The SAMHD1 tetramer structure provides the basis for a mechanistic understanding of its function in HIV restriction and the pathogenesis of AGS.

1 Goldstone, D. C. et al. HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase. Nature 480, 379-382, doi:10.1038/nature10623 (2011).
2 Yan, J. et al. Tetramerization of SAMHD1 is required for biological activity and inhibition of HIV infection. Journal of Biological Chemistry, doi:10.1074/jbc.M112.443796 (2013).

Structural Mechanism of Tat Binding to 7SK snRNA.

Victoria D'Souza

Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138

The transition from transcriptional initiation to elongation of the integrated HIV-1 genome is critical for expression of the complete viral genome. The viral Tat protein plays a central role in this process due to unique sequence and structural properties that allow interactions with multiple cellular and viral proteins and RNA partners. The molecular basis of this process has slowly emerged, mainly through the identification of the cellular small nuclear 7SK ribonucleoprotein (snRNP) complex, which sequesters the essential P-TEFb required for elongation. Tats role is to extract active P-TEFb from the 7SK snRNP by displacing the HEXIM proteins. We have focused on a key intermediate in this process: the highly specific interaction of the arginine-rich motif (ARM) of Tat with a conserved stem-loop in the 5' region of 7SK (7SK-SL1). My talk will detail the structural properties of this interaction, and describe a potential molecular mimicry between 7SK-SL1 and TAR, which allows the ARM of Tat to interact with both cellular and viral RNA, respectively.

Structural Studies of Host-vRNA Interactions Involved in HIV Genome Splicing

Blanton S. Tolbert

Department of Chemistry, Case Western Reserve University, Cleveland, OH

Regulated splicing events are necessary to express the full complement of HIV proteins and to establish pools of genomic RNA. Aberrations in the viral splicing machinery impair replication fidelity and in some cases render the virus less infectious. Thus, HIV splicing pathways represent novel targets for therapeutic intervention. There is significant evidence that splicing decisions are determined by the dynamic assembly/disassembly of *trans* host factors with *cis* viral RNA control elements; however, very little is known about the protein-RNA interactions and structures that determine whether a splice site will be activated or repressed. To fill this knowledge gap, we endeavor to develop quantitative models of HIV regulated splicing. In my talk, I will discuss our recent progress to elucidate the structural basis of HIV splice site selection by the host hnRNP A1 protein.

Computational and Experimental Approaches for Targeting Transient State Structures of HIV-1 RNA

Jang Hyun Lee, Loic Salmon, and Hashim M. Al-Hashimi

Department of Chemistry, University of Michigan, Ann Arbor, MI 48109

We describe the development and application of approaches that combine solution state NMR, X-ray crystallography, and computation for visualizing alternative fleeting structures of RNA that exist in low abundance (<1% population) and for very short periods of time (lifetimes ranging between microseconds and milliseconds). Computational, conventional high throughput screening, and NMR-directed screening approaches are then combined to identify small molecules that trap these alternative RNA conformations thereby disrupting RNA activity. We will describe progress in using these approaches in targeting the transactivation response element (TAR) and the stem-loop 1 (SL1) RNAs.

Proteomic and Structural Studies of HIV Budding and ESCRT-III Assembly

CHEETAH Center, Wesley I. Sundquist and Adam Frost

Department of Biochemistry, University of Utah, Salt Lake City, UT-84112-5650 USA

HIV-1 budding is facilitated by the host ESCRT (Endosomal Sorting Complex Required for Transport) pathway. The ESCRT pathway also provides membrane fission activities during the cellular processes of abscission, MVB vesicle formation, and shedding vesicle release. HIV-1 $p6^{Gag}$ contains two late domain motifs that interact directly with the early-acting ESCRT factors TSG101/ESCRT-I and ALIX, and thereby help recruit the pathway to the neck of the budding virus. Studies in our laboratory and elsewhere indicate that HIV-1 Gag must also contain at least one additional late domain activity because overexpression of the HECT ubiquitin E3 ligase, NEDD4L (NEDD4.2) stimulates the release of a "crippled" virus that lacks TSG101 and ALIX binding sites. The mechanism by which HIV-1 Gag recruits NEDD4L has not been clear, however, as there is no evidence that NEDD4L can bind Gag directly. We will describe our proteomic, biochemical, and functional studies that have identified a new host factor for HIV-1 budding factor, Angiomotin (AMOT), which functions early in the process and appears to function by linking Gag and NEDD4L.

An important outstanding question is how the ESCRT machinery draws the necks of budding viruses, vesicles, and intercellular bridges together. Studies from a number of laboratories have established that ESCRT-III family proteins form filaments that are intimately involved in these membrane remodeling processes. Although crystal structures are available for ESCRT-III proteins in their monomeric, autoinhibited conformations, the molecular architectures of polymeric ESCRT-III filaments have not yet been described. We will present a subnanometer resolution cryo-EM reconstruction of the helical filaments formed by the co-assembly of an ESCRT-III protein pair: IST1 and CHMP1B. The reconstruction reveals that IST1 and CHMP1B copolymerize into a novel paired, helical filament. We will also describe reconstitution experiments in which helical arrays of recombinant IST1 filaments bind and tubulate membranes with a topology that matches ESCRT-mediated membrane remodeling. Together, these experiments reveal how ESCRT-III subunits can form filaments, assemble into helices, and bind and remodel membranes.

HARC Center: HIV Accessory and Regulatory Complexes, Overview

Tom Alber[1,6], Yifan Cheng[2,6], Charles Craik[3,6], Jennifer Doudna[1,6], Alan Frankel[2,6], John Gross[3,6], Matt Jacobson[3,6], Nevan Krogan[4,6,7], Matija Peterlin[5] and Robert Stroud[2,6]

[1]Department of Molecular and Cell Biology, UC Berkeley, Berkeley, CA, [2]Department of Biochemistry and Biophysics, UCSF, San Francisco, CA, [3]Department of Pharmaceutical Chemistry, UCSF, San Francisco, CA, [4]Department of Cellular and Molecular Pharmacology, [5]Department of Medicine, UCSF, San Francisco, CA; [6]California Institute for Quantitative Biosciences, QB3, [7]J. David Gladstone Institutes, San Francisco, CA

The HARC Center has been taking a comprehensive "systems-to-structure" approach to defining the host interactome of HIV. Experiments conducted in two cell lines expressing each of the HIV proteins uncovered a large set of high-quality interactions that were previously unknown, which has led to an appreciation of considerable new virus-host biology. The establishment of a reliable pipeline to characterize protein-protein interactions from global mass spectroscopy to high-resolution structure has influenced all projects in the Center, which focuses on the roles of HIV accessory and regulatory proteins in three functions that are essential to the viral lifecycle: Restriction/Degradation (Vif, Vpu, Vpr & PR), Transcription (Tat) and RNA Trafficking and Translation (Rev). We have identified numerous cellular partners of these and other HIV proteins, and many have been shown to be essential for the viral lifecycle. In several cases, structural and functional studies have revealed unexpected protein interfaces with potential therapeutic implications. One such finding was the discovery that HIV Vif recruits the transcription factor, CBFb. This interaction stabilizes Vif, inhibits restriction and also broadly alters transcription in infected T cells. Functional and biochemical analyses of the Vif-CBFb interaction reveal how Vif simultaneously hijacks two pathways: cullin-RING ligase-mediated degradation and RUNX1 target gene transcription, with potential effects on T cell differentiation. In addition, we found that HIV Tat recruits the Super Elongation Complex (SEC) not only by binding the P-TEFb kinase, but also by making direct contacts with the flexible SEC scaffold protein. The crystal structure of a fragment of the AFF4 scaffold in complex with human P-TEFb reveals that the scaffold fills a site on Cyclin T1 adjacent to the Tat-binding site. This creates a deep pocket where Tat is positioned to contact both AFF4 and Cyclin T1. At the same time, significant inroads have been made into key complexes between other HIV proteins and cellular partners such as Rev-Crm1 complexes, where a negative-stain EM reconstruction shows the arrangement of the viral RNA nuclear export complex and how the Rev-RRE RNA complex is altered by host protein binding. Progress has been made on global analysis of HIV-dependent post-translational modifications and identification of potential novel restriction factors degraded by Vpu- or Vpr-dependent pathways, as well as identification of new PR substrates. We are developing experimental and computational methods to structurally and functionally interrogate these systems, including technological advances in cryo-EM, which provide access to dramatically higher resolution structures of small integral membrane proteins and small, heterogeneous soluble complexes, which are characteristic of many of the HIV-host assemblies. Overall, the HARC Center's studies of complexes with high-confidence human partners of accessory and regulatory proteins are opening rich new directions and providing fundamental insights into HIV and host biology.

Assembly and Molecular Architecture of the Vif E3 Ubiquitin Ligase

Dong Young Kim[1,4], Eunju Kwon[1,4], Paul Hartley[2,4], David Crosby[3], Sumanjit Mann[3], Nevan Krogan[2,4,5], and John Gross[1,4]

[1]Department of Pharmaceutical Chemistry, [2]Department of Cellular and Molecular Pharmacology, [3]Department of Biochemistry and Biophysics, UCSF, San Francisco, CA; [4]California Institute for Quantitative Biosciences, QB3, [5]J. David Gladstone Institutes, San Francisco, CA

Primate lentiviral Vif hijacks a cellular ubiquitin E3 ligase CRL5 to promote degradation of the APOBEC3 family restriction factors. This activity also requires a transcription cofactor CBFß, which promotes transcription of RUNX target genes in uninfected cells. We have shown that the reconstituted CRL5-Vif-CBFß holoenzyme is active at promoting ubiquitin chain synthesis on APOBEC3 family members in vitro. Here we present a low-resolution structure of the Vif E3 holoenzyme determined by small-angle X-ray scattering in solution. We compare the architecture of this Vif E3 to its cellular counterparts and discuss surfaces that are important for assembly and activity. We present several observations that indicate incorporation of CBFß into the Vif E3 precludes its association with RUNX transcription factors, exerting a global effect on synthesis of RUNX target genes. Our findings suggest a viral accessory protein can impact multiple cellular pathways by nucleating a single multi-protein complex. In this way, a pathogen with limited protein coding capacity may efficiently perturb host cell biology.

HIV Tat Recognition of Human Transcription Elongation Factors

Tom Alber[1], Katherine Bao[1], Seemay Chou[1], Charles Craik[2], Ivan D'Orso[3], Jennifer Doudna[1], Alan Frankel[3], Nanhai He[1], Matt Jacobson[2], Stefanie Jager[4], Nevan Krogan[4], Matija Peterlin[5], Ursula Schulze-Gahmen[1], Heather Upton[1], and Qiang Zhou[1]

[1]Department of Molecular and Cell Biology, UC Berkeley, Berkeley, CA, [2]Department of Pharmaceutical Chemistry, UCSF, San Francisco, CA, [3]Department of Biochemistry and Biophysics, UCSF, San Francisco, CA, [4]Department of Cellular and Molecular Pharmacology, [5]Department of Medicine, UCSF, San Francisco, CA

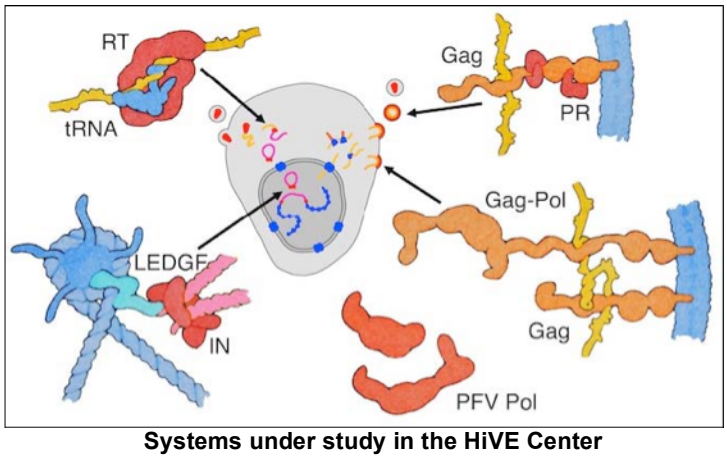
HIV Tat establishes a positive feedback loop for viral transcription not just by localizing the heterodimeric kinase, P-TEFb, to the viral promoter, but instead by recruiting P-TEFb as part of a large assembly of transcription elongation factors called the super elongation complex (SEC). The SEC activates transcription elongation by at least three different mechanisms. Here we present diverse collaborative approaches taken in the HARC Center to establish the architecture of the SEC and define the structural basis for Tat function. The homologous AFF1 and AFF4 proteins serve as a flexible scaffold with 30-50-residue binding sites for the other factors–including P-TEFb, ELL2 and ENL/AF9–distributed across the first 750 amino acids. Biochemical and biophysical studies revealed minimal hydrophobic binding sites in AFF4 that were slightly smaller than the functional binding sites defined in vivo using assays of SEC assembly and transcriptional stimulation. The crystal structure of the N-terminal segment of AFF4 in complex with P-TEFb showed that the scaffold protein bound in an extended conformation to the Cyclin T1 (CycT1) subunit without making contacts to the kinase subunit. Unexpectedly, the scaffold associated in position to directly interact with HIV Tat. A systematic alanine scan of Tat revealed that residues in the predicted AFF4 interface are essential for Tat activity in vivo. Also consistent with direct Tat-AFF4 contacts, the scaffold bound over an order of magnitude tighter to Tat-P-TEFb than to P-TEFb alone. Moreover, Tat expression rescued SEC assembly of diverse AFF4 variants with mutations in the P-TEFb interface. These results suggest a model in which HIV Tat recognizes an unexpected intersubunit pocket between AFF4 and CycT1. This pocket has features shared by drug binding sites, implicating the host SEC as a potential target for new antiviral therapeutics. To test this idea, we have designed novel cyclic peptides predicted to enter cells and inhibit Tat binding. Overall, these studies provide insights into the regulation of transcription elongation of HIV and host genes.

HIVE Center: HIV Interactions and Viral Evolution of Drug Resistance

Eddy Arnold[1], John Elder[2], Alan Engelman[3], M.G. Finn[4], Valery Fokin[5], Patrick Griffin[6], Stephen Hughes[7], Roger Jones[1], Mamuka, Kvaratskhelia[8], Ron Levy[1], Joe Marcotrigiano[1], Arthur Olson[9], Michael Parniak[10], Alan Rein[7], Dave Stout[9], Bruce Torbett[11]

[1]Department of Chemistry and Chemical Biology, Rutgers University; [2]Department of Immunology and Microbial Science, The Scripps Research Institute; [3]Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute; [4]Department of Chemistry and Biochemistry, Georgia Institute of Technology; [5]Department of Chemistry, The Scripps Research Institute; [6]Department of Molecular Therapeutics The Scripps Research Institute Florida; [7]Center for Cancer Research, National Cancer Institute, NIH; [8]College of Pharmacy, The Ohio State University; [9]Department of Integrative Structural and Computational Biology, The Scripps Research Institute; [10]Department of Microbiology & Molecular Genetics, University of Pittsburgh; [11]Department of Molecular and Experimental Medicine, The Scripps Research Institute

The HIVE Center is focused on the structural and dynamic interactions of the major HIV enzymes, reverse transcriptase/RNAse H, protease and integrase, with their molecular partners and effectors in key processes of the viral life cycle. By studying how the structures of the HIV polyprotein precursors direct assembly, maturation and replication, as well as how HIV-Host interactions drive DNA replication and integration, we explore how therapeutic targeting impacts the evolution of drug resistance and what the structural and dynamic consequences of resistance mutations are on the HIV life cycle. This approach is significant because of the promise of new structural insights into the interdependence of viral mechanisms and the potential for new drug design methodologies and therapeutic strategies.



Systems under study in the HiVE Center

This overview will present the nature of the collaborations and research within the HIVE Center that have developed within the first year of the its existence, with selected examples of work that highlight the broad range of techniques and studies that are being undertaken, from fragment-based discovery of novel allosteric inhibitors to viral population genetics of patients under anti-retroviral therapies.

Comparative Experiences in Crystallographic Fragment Screening with HIV-1 Reverse Transcriptase and Integrase

Eddy Arnold

Center for Advanced Biotechnology and Medicine, Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, New Jersey

Reverse transcriptase (RT) and integrase (IN) are crucial drug targets for treatment of HIV. The HIV-1 RT p66/p51 heterodimer undergoes many conformational changes during the replication of the HIV-1 genome. Recent advances in the crystallization of RT by protein engineering have generated crystals that diffract X-rays to better than 2.0 Å resolution. The crystals allowed for the systematic application of fragment-based drug discovery by X-ray crystallography to be applied to RT. Fragment-based drug discovery involves the screening of a highly diverse small molecule (<300 Da) library to identify relatively weak binding compounds that can serve as platforms for lead design. HIV-1 RT crystals have been screened with more than 750 drug-like fragments and structures were solved by X-ray crystallography with typical resolution ranging between 1.8 and 2.5 Å. Fragments have been found to bind at numerous sites throughout the heterodimer (Bauman, Patel, *et al.*, J. Med. Chem. **56**:2738-2746). Several new allosteric inhibitory sites, in both the polymerase and RNase H regions, have been discovered through these efforts.

Crystals of the catalytic core domain (CCD) of HIV-1 IN have recently been screened with 950 drug-like fragments. The crystals diffract to a typical resolution of 1.7 Å resolution and are relatively robust when soaked with cocktails Only one compound out of 950 was observed to bind to the HIV-1 IN CCD, in a region of the structure close to the LEDGF-binding site. The paucity of hits may reflect the lack of binding pockets and grooves in the IN CCD relative to the large, complex, and conformationally flexible HIV-1 RT. The HIV-1 IN project is being conducted in collaboration with the groups of Mamuka Kvaratshelia and James Fuchs at Ohio State University and Alan Engelman at Harvard University. We are grateful to NIGMS for support of the HIVE P50 Center (GM103368; Center Director is Arthur Olson, The Scripps Research Institute).

Allosteric Inhibitors Deregulate Integrase Multimerization During HIV-1 Particle Maturation

Kellie A. Jurado[1], Hao Wang[1], Alison Slaughter[2], Lei Feng[2], Jacques J. Kessl[2], Yasuhiro Koh[1], Weifeng Wang[1], Allison Ballandras-Colas[1], Pratiq A. Patel[3], James R. Fuchs[3], Mamuka Kvaratskhelia[2], and Alan Engelman[1]

[1]Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute and Department of Medicine, Harvard Medical School, Boston, MA 02215; [2]Center for Retrovirus Research and Comprehensive Cancer Center and [3]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210

Integration is essential for HIV-1 replication, and the viral integrase (IN) protein is an important therapeutic target. Allosteric IN inhibitors (ALLINIs) that engage the IN dimer interface at the host factor LEDGF/p75 binding site are an emerging class of small molecule antagonists. Consistent with the inhibition of a multivalent drug target, ALLINIs display steep antiviral dose response curves. ALLINIs multimerize IN protein and concordantly block its assembly with viral DNA in vitro, indicating that the disruption of two integration-associated functions, IN catalysis and the IN-LEDGF/p75 interaction, determines the multimodal mechanism of ALLINI action. We now demonstrate that ALLINI potency is unexpectedly accounted for during the late phase of HIV-1 replication. The compounds promote virion IN multimerization and, reminiscent of pleiotropic class II IN mutations, block the formation of the electron-dense viral core and inhibit reverse transcription and integration in subsequently infected target cells. Mature virions are recalcitrant to ALLINI treatment, and compound potency during virus production is independent of the level of LEDGF/p75 expression. We conclude that cooperative multimerization of IN by ALLINIs combined with the inability for LEDGF/p75 to effectively engage the virus during its egress from cells underscores the multimodal mechanism of ALLINI action. Our results highlight the versatile nature of allosteric inhibitors to primarily inhibit viral replication at a step that is distinct from the catalytic requirement for the target enzyme. The vulnerability of IN to small molecules during the late phase of HIV-1 replication unveils a pharmacological Achilles' heel for exploitation in clinical ALLINI development.

HIV-1 Gag Protein: The Role of Oligomerization in Virus Assembly

S.A.K. Datta, Ina P. O'Carroll, and Alan Rein

HIV Drug Resistance Program, Center for Cancer Research, National Cancer Institute, Frederick, MD

HIV-1 Gag is a multi-domain protein, containing the major domains matrix, capsid (CA), nucleocapsid (NC), and p6, plus two short "spacers", SP1 between CA and NC and SP2 between NC and p6. We have produced Gag protein in *E.coli*; it differs from authentic Gag in lacking the myristate modification at its N-terminus and the p6 domain at its C-terminus. It is a soluble protein, but assembles into virus-like particles (VLPs) upon addition of nucleic acid (NA).

How does NA-binding contribute to VLP assembly? It is significant that the NAs can be quite short (25-30 bases long), capable of binding only a few Gag molecules. A key observation is that a chimeric Gag protein, in which the NC domain (the primary NA-binding domain) has been replaced by a leucine-zipper domain, can still assemble efficiently; the resulting VLPs do not contain NAs. The fact that NC can be replaced by a dimer-forming leucine zipper suggests that the role of NAs in assembly is to promote oligomerization; Gag molecules that are associated in oligomers can evidently proceed to assemble into VLPs.

We have analyzed a peptide representing the last 8 residues of CA and the first 10 residues of SP1. The sequence of this stretch of Gag could form an amphipathic helix, but it is unstructured in crystal or NMR analyses of proteins containing these residues. Circular dichroism data shows that the peptide can adopt a helical conformation in the presence of organic solvents. Most importantly, its conformation in aqueous media is concentration-dependent: it is a coil at low concentration, but becomes helical at high concentration. Presumably, if the concentration is high enough, this stretch folds into a helix and multiple helices associate with each other, burying hydrophobic residues. Perhaps this concentration-dependent change in conformation can occur within Gag and can be propagated into the CA domain, exposing new residues for Gag-Gag interaction leading to VLP assembly. This change could explain why oligomerization of Gag seems to be a prerequisite for assembly. Current efforts in the laboratory are directed toward determining the atomic structure of SP1 in such a bundle.

One way that a high local concentration of Gag molecules might be achieved within the cell is by binding to the plasma membrane; another is by cooperative binding to RNA; yet another is by direct interaction via the dimer interface within CA. We have generated Gag mutants in which each of these functions is disrupted; overexpression of these mutants in mammalian cells still permits some assembly of structures similar to VLPs. However, Gag with any two of these mutations is incapable of assembly under our conditions. These observations suggest that Gag can use alternative means to reach the high local concentration necessary for assembly.

This work was supported by the Intramural Research Program of the NIH, National Cancer Institute and Center for Cancer Research.

A Personal History Of Structural Virology

Michael G. Rossmann

Department of Biological Sciences, Purdue University, West Lafayette, IN, 47907

I will chronicle the growth of structural knowledge on viruses and its impact on manipulating the properties of viruses for human benefit as seen through my own experience. I will follow this theme in the investigation of plant viruses such as Tobacco Mosaic Virus, small RNA icosahedral viruses and small RNA icosahedral picornaviruses. Although the initial structural investigations of viruses was dependent primarily on X-ray crystallography, more recent work has required a combination of electron microscopy for viewing the whole virus and X-ray crystallography for determining the structure of the viral components as well as other physical tools. These hybrid methods have been particularly useful in the study of lipid envelope viruses such as alpha and flavi viruses. In addition hybrid techniques have been essential for the study of complex bacterial viruses and their efficient infection mechanisms. Most recently cryo electron tomography has been used to study pleomorphic viruses such as the mumps and measles orthomyxoviruses that had previously been somewhat out of range for detailed structural investigations.

Introduction to the Program Project on Structure-Based Antagonism of HIV-1 Envelope Function in Cell Entry

Irwin M. Chaiken[1], Ernesto Freire[2], Wayne A. Hendrickson[3], Judith M. LaLonde[4], Walther Mothes[5], Amos B. Smith III[6], Joseph G. Sodroski[7]

[1]Drexel University College of Medicine, [2]Johns Hopkins University, [3]Columbia University, [4]Bryn Mawr College, [5]Yale University, [6]University of Pennsylvania, [7]Dana-Farber Cancer Institute

The overarching goal of this Program is to determine the vulnerabilities of the HIV-1 Env protein entry machine as a target for disease intervention by identifying Env inhibitors, defining their structural mechanisms of action, and using a structural/mechanistic framework as a guide to optimize antagonist functions.

Inhibition of the initial entry of HIV-1 into host cells remains a compelling, yet elusive means to prevent infection and spread of the virus. Inhibitors of HIV-1 Env that can either block cell interactions, inactivate the trimeric virus spike protein complex before receptor encounter or disrupt receptor-induced conformational changes in the Env would hold great promise of inhibiting initial HIV-1 infection. Such inhibitors would provide virus-targeted molecular weapons both to prevent AIDS transmission, a global health priority, and to treat already-infected individuals.

In spite of the great potential of Env inhibitors for AIDS prevention and treatment, progress has been slowed by: [1] lack of understanding of the conformational dynamics and allosteric mechanisms that control receptor-mediated interactions and activation of the Env proteins leading to cell entry; and [2] lack of knowledge of specific sites and residues in the Env complex, including in the context of the trimeric spike protein complex, that need to be targeted for optimal competitive and allosteric inhibition. These factors have constrained our ability to design effective molecular agents that target the virus surface as a means to prevent infection. Nonetheless, the efforts of our Program Project have led to the discovery of two classes of Env gp120 inhibitors that utilize the highly conserved CD4 binding site but with very different modes of action. Investigation of these inhibitors has defined unique pathways to specifically engage the virus Env trimer and cause both inactivation of the virus and blockade of virus entry into the host cell. Going forward, our Program Project seeks to take advantage of these new results through state-of-the-art structure- and mechanism-based approaches achieved by the collaboration of a multi-institutional team with expertise in high-resolution structure determination, structural dynamics, kinetic, thermodynamic and structural mechanisms of protein-protein interactions, chemical design and synthesis, computational methods and virology. We will apply this team approach to structure-based design and mechanistic investigations of the inhibitor chemotypes that we have already developed, as well as of new inhibitor chemotypes as they are discovered in our own and other laboratories. Overall, the Program Project aims to provide a broad-based research infrastructure to identify new paths for the discovery of preventive and therapeutic agents that interact with Env proteins, inactivate the virus in advance of cell encounter and block infections of host cells.

Molecular Architecture of the Uncleaved HIV-1 Envelope Glycoprotein Trimer

Youdong Mao[1], Liping Wang[1], Christopher Gu[1], Alon Herschhorn[1], Anik Désormeaux[2], Andrés Finzi[2], Shi-Hua Xiang[3] & Joseph Sodroski[1,4,5]

[1]Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA 02115. [2]Centre de Recherche du CHUM, Department of Microbiology and Immunology, Université de Montréal, Montréal, Québec H3A 2B4, Canada. [3]Nebraska Center for Virology, School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE 68583. [4]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA 02139. [5]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA 02115.

The human immunodeficiency virus (HIV-1) envelope glycoprotein (Env) trimer, a membrane-fusing machine, mediates virus entry into host cells and is the sole virus-specific target for neutralizing antibodies. Binding the receptors, CD4 and CCR5/CXCR4, triggers Env conformational changes from the metastable unliganded state to the fusion-active state. We used cryo-electron microscopy to obtain a 6-Å structure of the membrane-bound, heavily glycosylated HIV-1 Env trimer in its uncleaved and unliganded state. The spatial organization of secondary structure elements reveals that the unliganded conformations of both gp120 and gp41 subunits differ from those induced by receptor binding. The gp120 trimer association domains, which contribute to interprotomer contacts in the unliganded Env trimer, undergo rearrangement upon CD4 binding. Intersubunit interactions maintain the gp41 ectodomain helical bundles in a “spring-loaded” conformation distinct from the extended helical coils of the fusion-active state. Quaternary structure regulates the virus-neutralizing potency of antibodies targeting the conserved CD4-binding site on gp120. The Env trimer architecture provides mechanistic insights into the metastability of the unliganded state, receptor-induced conformational changes, and quaternary structure-based strategies for immune evasion.

Temporal Definition of HIV-1 gp120 Structural Motions in Functional Envelope Spikes

James B. Munro[1], Jason Gorman[2], Jonathan Stuckey[2], Peter D. Kwong[2], Scott C. Blanchard[3] & Walther Mothes[1]

[1] Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, Connecticut 06536, USA; [2] Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA; [3] Department of Physiology & Biophysics, Weill Cornell Medical College of Cornell University, New York, New York 10021, USA.

HIV-1 entry is mediated by the envelope spike (Env), a fusion machine composed of gp120 and gp41 subunits, and activated by receptors CD4 and coreceptor. Although the overall architecture of the spike and crystal structures of individual subunits have been determined, real-time Env motions have not been characterized. Here we show the HIV-1-spike of strain NL4-3 in its unliganded state is structurally dynamic and intrinsically samples at least three distinct conformations. We introduced probes into discrete domains of gp120 which enabled time-resolved monitoring of inter-domain distances though single-molecule fluorescence resonance energy transfer (smFRET) measurements of infectious virus. smFRET trajectories displayed selective transitions, with the conformation corresponding to the CD4-bound state a preferred intermediate. Distances derived from smFRET were integrated with known structures to produce a temporally accurate simulation of the outer domain of gp120, as it locks and unlocks spike-fusion machinery through dynamic sampling of ground and activated states. smFRET imaging now allows determination of the conformational consequences of HIV-1 Env antagonism by broadly neutralizing antibodies and small-molecule inhibitors.

HIV Envelope Glycoprotein Structure and Mechanisms of Viral Entry

Sriram Subramaniam

Laboratory of Cell Biology, National Cancer Institute, Bethesda, MD

We are using emerging tools in 3D electron microscopy and image processing to analyze the molecular structures of unliganded and antibody-bound trimeric HIV-1 Env in native and solubilized forms, and to explore cellular mechanisms involved in HIV entry. These studies are providing new insights into the structural diversity of Env and the strain-dependent variations in the structural consequences of ligand binding by Env. Further, comparative analysis of the conformational states stabilized by different antibodies bound to Env provides structural snapshots of the changes in molecular architecture that result from activation of Env by binding to different ligands. 3D visualization of cell-cell virus transfer at virological synapses is providing surprising and new insights into the mechanisms underlying effective cell-to-cell virus transmission. I will discuss these and related topics in my talk.

Antibody-Stabilized Env Structures and Vaccine-Design Implications

Peter D. Kwong

Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health

Type 1 fusion machines such as the viral spike of HIV-1 or the fusion glycoprotein of respiratory syncytial virus (RSV) assume multiple conformations, related to their function in merging virus and target cell membranes during entry. With HIV-1, the viral spike and its subunits, gp120 and gp41, assume additional conformations related to immune evasion, with shed Env subunits exposing surfaces and assuming conformations which are inaccessible on the functional viral spike. By contrast, Env structures recognized by broadly neutralizing antibodies potentially delineate sites and conformations of vulnerability, which might be suitable vaccine targets. Here we describe crystallographic structures of Env bound by broadly neutralizing antibodies and detail implications for vaccine design. We show that the potent RSV-neutralizing antibody D25 locks the fusion glycoprotein of RSV in its prefusion conformation and that structure-based stabilization of this particular conformation results in high titers of RSV-neutralizing activity. We show that the highly effective HIV-1-neutralizing antibody, PG9, binds to a D25-equivalent site on the HIV-1-viral spike and propose that structure-based stabilization of the PG9 epitope will allow for the elicitation of broadly neutralizing antibodies. Together the results outline a structure-based paradigm for vaccine design.

An Integrative Approach to Characterizing Broadly Neutralizing Antibodies to the HIV-1 Glycan Shield

Ian A. Wilson, D.Phil.

Department of Integrative Structural and Computational Biology, Skaggs Institute for Chemical Biology, CHAVI-ID, and IAVI Neutralizing Antibody Center, The Scripps Research Institute, La Jolla, CA 92037 USA. Email: wilson@scripps.edu

The discovery and isolation of highly potent, broadly neutralizing antibodies (bnAbs) that recognize a broad diversity of HIV-1 isolates has opened up tremendous opportunities for enhancing our understanding of how HIV-1 can be neutralized. Recently, a number of human monoclonal antibodies have been isolated that potently neutralize HIV-1 isolates across all clades. Structural characterization of these bnAbs by X-ray and EM has led to the identification of novel epitopes on HIV-1 Env as well as unique features that enable the antibodies to penetrate the glycan shied and bind epitopes that consist of glycans and segments of gp120. The structural and biophysical studies have suggested varied mechanisms of neutralization. Elucidation of the structure and function of these bnAbs has now provided valuable insights that can be used for structure-assisted vaccine design.

This work was done in collaboration with the labs of Andrew Ward, John Moore and Dennis Burton and was supported by NIH grants UM1 AI100663 (the Scripps Center for HIV/AIDS Vaccine Immunology and Immunogen Design (CHAVI-ID), P01 AI082362 (HIVRAD) and R01 AI084817, and the International AIDS Vaccine Initiative (IAVI).

The Interdependency of Drug Resistance Evolution and Drug Design: HIV-1 Protease a Case Study

Celia Schiffer

University of Massachusetts Medical School

HIV-1 protease inhibitors are the most potent antiretroviral drugs for the treatment of HIV-AIDS. These drugs are ideal for therapy as they target the viral protease responsible for viral maturation and thus the spread of the virus. Unfortunately due to the rapid evolution of HIV both around the world and within patients, coupled with the selective pressure of therapy, viable multidrug resistant variants arise. Drug resistance at the molecular level is a subtle change in the balance of recognition events between the relative affinity of the enzyme to bind inhibitors and its ability to bind and cleave substrates. In many HIV-1 protease variants, multiple site mutations co-evolve to both decrease the affinity of a particular inhibitor and increase the viability and fitness of the enzyme. We investigate the impact of particular mutations on conferring drug resistance as not being simply additive, but that these mutations have a complex interdependent effect leading to viable variants that are highly resistant to existing drugs.

The Impact of Alterations in Enzyme Dynamics in Drug Resistance

Rieko Ishima

University of Pittsburgh

One possible mechanism by which mutations contribute to drug resistance is through altering enzyme dynamics. Dynamics of HIV protease is crucial for the protein's biological activity. The flaps covering the active site need to "open", to allow ligand access, and to "close" once the ligand is bound. The dynamics of the flaps are intimately related to the kinetic balance between substrate processing versus inhibition by a drug molecule. Our previous NMR experiments revealed that the dynamics of the flaps are highly complex, with samplings of various conformational states in solution over a wide range of time scales. In this project, we aim to eluicdate the effect of drug resistance mutations on the dynamics of PR and how dynamics relates to inhibitor affinity, thermodynamics of inhibitor binding, and drug resistance. The effect of drug resistance mutations on protease dynamics likely involves: 1) alterations in the flap dynamics, 2) alterations in the core dynamics, and 3) coupled changes between these two regions.

Tracking Evolution of Drug Resistance for Highly Potent HIV Protease Inhibitors

Ronald Swanstrom

University of North Carolina, Chapel Hill – School of Medicine

As protease inhibitors become more potent, resistance mutations become more complex. There is an increasing need to understand resistance mutations that arise outside of the protease, a continuing need to understand the interplay of resistance mutations within the protease, and an opportunity to explore the implications of tight binding transition state analog inhibitors with increasing potency. The evolution of resistance under conditions of increasing inhibitor concentration represents a wealth of information about the relative contribution of different mutations at varying inhibitor concentrations, the pathways to the addition of multiple mutations, and the replacement of one lineage of resistance by another. We are characterizing these using deep sequencing techniques. As inhibitor potency has increased, such as seen in Darunavir (DRV), the range of mutations selected increasingly include mutations outside of the protease coding domain. As the inhibitors become even more potent we predict that resistance paradoxically will become weaker and even more in the form of mutations mostly affecting fitness both within and outside of the protease active site.

Temporal Tracking of Acquisition of Resistance and Integration of Complex Datasets

Konstantin Zeldovich

University of Massachusetts Medical School

Rapid development of drug resistance and multidrug resistance is a major problem for treating HIV infected patients. Resistant variants have been discovered against most if not all FDA-approved HIV-1 protease inhibitors, and resistance continues to evolve. The next level of understanding of this complex evolution requires knowledge of the temporal evolutionary pathways, in addition to traditional studies of evolved resistant variants and data acquired from EMPIRIC targeted libraries. Data from Influenza shows the powerful results of such an approach. Rationalization of the temporal aspect of acquiring resistance in viruses has important implications for successful design of novel antivirals, active against a wide spectrum of mutant targets while limiting further development of resistance.

Cryo-Electron Tomography Studies of HIV Assembly and Maturation

John Briggs

European Molecular Biology Laboratory, Meyerhofstraße 1, 69117 Heidelberg, Germany

The assembly of HIV-1 is driven by oligomerization of the major structural protein, Gag. Gag is a multi-domain polyprotein including three conserved folded domains, MA (matrix), CA (capsid) and NC (nucleocapsid). Assembly of an infectious virion proceeds in two stages. In the first, Gag oligomerization at the plasma membrane leads to the budding of an immature, non-infectious virus particle within which Gag forms an incomplete, roughly spherical shell. In the second, proteolytic cleavage of Gag separates it into its component domains, leading to rearrangement of the viral structure into the mature infectious form.

To obtain a higher-resolution understanding of the arrangement of Gag in the immature virion, we have applied cryo-electron microscopy methods to study the arrangement of Mason-Pfizer monkey virus Gag in immature-like tubular arrays. These data reveal interfaces mediating Gag assembly, and the structural rearrangements associated with maturation.

We have also applied cryo-electron microscopy and tomography to study HIV-1 virions in the immature and mature forms, as well as a panel of mutant virus particles defective for processing at individual cleavage sites. These data shed light on the influence of individual cleavage sites on the progress of viral maturation.

Recent Progress in the Development of Potent and Broadly Active HIV-1 Maturation Inhibitors

Emiko Urano[1], Sherimay Ablan[1], Kayoko Waki[1], David E. Martin[2], T. J. Nitz[2], Carl T. Wild[2], and Eric O. Freed[1]

[1]Virus-Cell Interaction Section, HIV Drug Resistance Program, NCI-Frederick, Frederick, MD 21702; [2]DFH Pharma, Inc., Gaithersburg, MD

A betulinic acid-based compound, bevirimat (BVM), the first-in-class HIV-1 maturation inhibitor, acts by blocking a late step in protease-mediated Gag processing: the cleavage of the capsid-spacer 1 (CA-SP1) intermediate to mature CA. A structurally distinct molecule, PF-46396, was found to have a similar mode of action to that of BVM. Despite their similar mechanism of action, our work has revealed significant differences in the target of these two compounds. While BVM resistance maps to residues surrounding the CA-SP1 cleavage site, selections in PF-46396 identified resistance mutations clustered at two additional regions far upstream in CA: the major homology region (MHR) and CA amino acid 201. Interestingly, a group of these MHR mutants are profoundly PF-46396-dependent in Gag assembly/release and virus replication. Biochemical analyses revealed that these mutants are defective for Gag multimerization in the absence of PF-46396, but that proper assembly is triggered by the addition of the compound. The severe defect exhibited by the inhibitor-dependent MHR mutants in the absence of the compound can also be corrected by second-site compensatory changes in SP1, suggesting structural and functional cross-talk between the MHR in CA and SP1.

BVM was shown in multiple clinical trials to be safe and effective in reducing viral loads in HIV-1-infected patients. However, a single-amino-acid polymorphism in the SP1 region of Gag (SP1-V7A) lead to variable patient response in BVM-treated patients. The reduced susceptibility of SP1-V7A polymorphic HIV-1 to BVM led to the discontinuation of its clinical development. We carried out an extensive medicinal chemistry campaign coupled with analysis of HIV-1 replication kinetics, single-cycle infectivity, and CA-SP1 processing and identified a set of BVM derivatives that are not only more potent than BVM against WT HIV-1 but also show robust antiviral activity against SP1-V7A and other CA-SP1 region polymorphs. The best of these analogs displayed ~3-log improved potency against V7A relative to BVM. Selection experiments identified a panel of resistant mutants that have been analyzed for their effect on viral assembly and fitness and CA-SP1 processing efficiency. Collectively, these results provide novel insights into the structure of the maturation inhibitor-binding site and the role of the CA MHR and SP1 in virus assembly and maturation. These findings also offer valuable insights into ongoing efforts to develop maturation inhibitors as a new class of therapeutically effective antiretroviral.

Structure of the Rhesus Monkey TRIM5α SPRY Domain and Its Interactions with the HIV Capsid

Nikolaos Biris[1], Dmytro Kovalskyy[1], Alexander B. Taylor[1], Andrei Tomashevski[1], P. John Hart[1], Dmitri N. Ivanov[1]

[1]Department of Biochemistry and Cancer Therapy and Research Center, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229

TRIM5α restriction factors bind to the capsid protein of the retroviral core and block retroviral replication. Affinity of TRIM5α for the capsid is a major host tropism determinant of HIV and other primate immunodeficiency viruses, but the molecular interface involved in this host-pathogen interaction remains poorly characterized. We use X-Ray Crystallography, NMR and other biophysical approaches to characterize the structure the rhesus TRIM5α SPRY domain and its interactions with a selection of HIV capsid constructs. The predicted extensive capsid binding interface maps on the structurally divergent face of SPRY formed by hyper-variable loop segments, confirming that binding specificity is a major determinant in SPRY evolution. The data are consistent with an interaction model in which one PRYSPRY domain binds more than one capsid monomer within the assembled retroviral core. The highly mobile SPRY v1 loop appears to span the gap between neighboring capsid hexamers making inter-hexamer contacts critical for restriction. The interaction interface is extensive, involves mobile loops and multiple epitopes, and lacks interaction hot spots—unique properties that make TRIM5α remarkably resistant to capsid mutations. These properties also result in a relatively low affinity of the isolated SPRY domain for the capsid, and TRIM5α-mediated restriction depends on the avidity effect arising from the self-association of TRIM5α. The capsid recognition mechanism of TRIM5α thus parallels antigen recognition by IgM antibodies, a similarity that may help explain some of the unusual functional properties of TRIM5α and refine the way we study TRIM5α-capsid interactions.

Critical Interactions of APOBEC3s: Molecular Approaches to Novel HIV Therapies

Reuben S. Harris[1], Hiroshi Matsuo[1], Celia A. Schiffer[2], Yuri Lyubchenko[3], and Moshe Kotler[4]

[1]Department of Biochemistry, Molecular Biology, and Biophysics, Institute for Molecular Virology, University of Minnesota, Minneapolis, Minnesota, USA; [2]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, 364 Plantation Street, Worcester, Massachusetts, USA; [3]Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, 986025 Nebraska Medical Center Omaha, Nebraska, USA; [4]Department of Pathology, Hebrew University-Hadassah Medical School, Jerusalem, Israel

The APOBEC3(A3)-Vif interaction dictates whether HIV 'lives' or 'dies'. In a normal infection, HIV uses its auxiliary protein Vif to neutralize the cellular retroviral restriction factors A3G and A3F. Conversely, in the absence of Vif, these A3 proteins are able to potently inhibit HIV infectivity. The A3-Vif interaction has therefore become a prime target for the development of novel therapeutic interventions. However, a current impediment to therapeutic development is a knowledge gap owing to the fact that there are few structural, biophysical and biochemical studies on these A3 proteins or HIV Vif. To address this gap, we have assembled a multidisciplinary team that combines the strengths of five laboratories, and we are working to achieve the following broad, high-impact objectives: (i) elucidate the full-length A3G structure and gain a comprehensive understanding for how this protein oligomerizes during HIV restriction, (ii) define how A3G binds single-stranded DNA and catalyzes cytosine to uracil deamination, and (iii) dissect the Vif interaction surfaces of A3G and A3F to fully understand critical similarities and differences (*e.g.*, 1-4). We are also developing novel A3G inhibitors and small peptides to use as molecular probes to dissect stages of the DNA deamination mechanism (*e.g.*, 5). Each investigator is applying his/her specific expertise to each of these aims and our team is working toward building-up a more comprehensive understanding of how A3F and A3G mediate HIV-1 restriction and how Vif counteracts these multifaceted and potent innate immune defenses. We anticipate that this knowledge will help accelerate the development and implementation of novel HIV/AIDS therapeutics that work by leveraging the A3/Vif axis.

1. Refsland, E.W., J.F. Hultquist & R.S. Harris (2012) Endogenous origins of HIV-1 G-to-A hypermutation and restriction in the nonpermissive T cell line CEM2n. *PLoS Pathogens*, 8(7): e1002800.
2. Shlyakhtenko, L.S., A.Y. Lushnikov, A. Miyagi, M. Li, R.S. Harris & Y.L. Lyubchenko (2012) Nanoscale structure and dynamics of ABOBEC3G complexes with single-stranded DNA. *Biochemistry* 51:6432-40.
3. Albin, J.S., J.S. Anderson, J.R. Johnson, E. Harjes, H. Matsuo, N.J. Krogan & R.S. Harris (2013) Dispersed sites of HIV Vif-dependent polyubiquitination in the DNA deaminase APOBEC3F. *Journal of Molecular Biology* 425:1172-1182.
4. Bohn, M.F., S.M.D. Shandilya, J.S. Albin, T. Kouno, B.D. Anderson, R.M. McDougle, M.A. Carpenter, A. Rathore, L. Evans, A.N. Davis, J. Zhang, Y. Lu, M. Somasundaran, H. Matsuo, R.S. Harris & C.A. Schiffer (2013) Crystal structure of the HIV-1 Vif-binding, catalytically active domain of the DNA cytosine deaminase APOBEC3F. *Structure*, *in press.*
5. Harjes, S., W.C. Solomon, M. Li, K.M. Chen, E. Harjes, R.S. Harris & H. Matsuo (2013) Impact of H216 on the DNA binding and catalytic activities of the HIV restriction factor APOBEC3G. *Journal of Virology*, *in press*.
6. Olson, M.E., M. Li, R.S. Harris & D.A. Harki (2013) Small molecule APOBEC3G DNA cytosine deaminase inhibitors based on a 4-amino-1,2,4-triazole-3-thiol scaffold. *ChemMedChem*. 8:112-7.

APOBEC3 Multimerization Correlates with HIV-1 Packaging and Restriction Activity in Living Cells

Yan Chen[1,3], Jinhui Li[1,3], Ming Li[1,2], Michael A. Carpenter[1,2], Rebecca McDougle[1,2], Patrick J. Macdonald[1,3], Reuben S. Harris[1,2], Joachim D. Mueller[1,3]

[1]School of Physics and Astronomy, [2]Department of Biochemistry, Molecular Biology and Biophysics, [3]Institute for Molecular Virology, University of Minnesota, Minneapolis, MN 55455

APOBEC3G belongs to a family of DNA cytosine deaminases that are involved in the restriction of a broad number of retroviruses including HIV-1. Many studies have identified two distinct mechanistic steps in Vif-deficient HIV-1 restriction: packaging into virions and deaminating viral cDNA. APOBEC3A, for example, although highly active, is not packaged and is therefore not restrictive. APOBEC3G, on the other hand, although having much weaker enzymatic activity, is packaged into virions and is strongly restrictive. Although many prior studies have described the propensity for APOBEC3 oligomerization, none have correlated this activity with HIV-1 restriction. Here, we address this problem by examining APOBEC3 oligomerization in living cells by molecular brightness analysis. We find that APOBEC3G forms high-order multimers as a function of protein concentration. APOBEC3B/D/F/H show similar multimerization capabilities. In contrast, APOBEC3A/C and APOBEC2 are monomers at all tested concentrations. A single amino acid substitution of C97A strongly decreases APOBEC3G's ability to multimerize and package into virions. These results combine to indicate that APOBEC3G multimerization may be required for the encapsidation stage of retrovirus restriction.

APOBEC3G Oligomerization State and Dynamics Assessed by Atomic Force Microscopy

Luda S. Shlyakhtenko[1], Alexander Y. Lushnikov[1], Atsushi Miyagi[1], Ming Li[2], Reuben S. Harris[2] and Yuri L. Lyubchenko[1]

[1]Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center; 986025 Nebraska Medical Center, Omaha, NE; [2]Department of Biochemistry, Molecular Biology, and Biophysics, Institute for Molecular Virology, Center for Genome Engineering, Masonic Cancer Center, University of Minnesota, 321 Church Street S.E., 6-155 Jackson Hall, Minneapolis, MN

The DNA cytosine deaminase APOBEC3G (A3G) is a two-domain protein that interacts with single-stranded DNA (ssDNA) with its N-terminal domain and catalyzes deamination with its C-terminal domain. A3G is considered an innate immune effector protein, with a natural capacity to block the replication of retroviruses such as HIV and retrotransposons. However, knowledge about its biophysical properties and mechanism of interaction with DNA are still limited. Oligomerization is one of these unclear issues. What is the stoichiometry of the free protein? What are the factors defining the oligomeric state of the protein? How does the protein oligomerization change upon DNA binding? How stable are protein oligomers? We address these questions here using atomic force microscopy (AFM) to directly image A3G protein in a free-state and in complexes with DNA, and using time-lapse AFM imaging to characterize the dynamics of A3G oligomers[1-2]. We have recently developed hybrid DNA substrates in which ssDNA substrate is flanked by one or two double-stranded fragments, enabling us to unambiguously identify complexes with ssDNA. We found that the formation of oligomers is an inherent property of A3G and that the yield of oligomers depends on the protein concentration. Concentrations of A3G as low as 2 nM, result in a primarily monomeric form. The protein changes it oligomerization state to predominantly dimers at 8 nM. Oligomerization of A3G in complexes with ssDNA follows the same pattern: the higher the protein concentrations the larger oligomers sizes. The specificity of binding to ssDNA does not depend on the A3G stoichiometry. The binding of large A3G oligomers requires a long ssDNA substrate; therefore, much smaller oligomers form complexes with short ssDNA, 9 nucleotides in length, than longer strands, such as 69 nt ssDNA. A3G oligomers dissociate spontaneously into monomers and this process primarily occurs through the monomer dissociation pathway, suggesting that different segments of A3G are involved in protein-protein and protein-DNA interactions.

References:

1. Shlyakhtenko, L. S., Lushnikov, A. Y., Li, M., Lackey, L., Harris, R. S. & Lyubchenko, Y. L. (2011). Atomic force microscopy studies provide direct evidence for dimerization of the HIV restriction factor APOBEC3G. *The Journal of Biological Chemistry* **286**, 3387-95.
2. Shlyakhtenko, L. S., Lushnikov, A. Y., Miyagi, A., Li, M., Harris, R. S. & Lyubchenko, Y. L. (2012). Nanoscale structure and dynamics of ABOBEC3G complexes with single-stranded DNA. *Biochemistry* **51**, 6432-40.

Crystal Structure of the Catalytic Domain of APOBEC3F Reveals a Novel Interaction Surface with Implications for Inhibitor Design and HIV-1 Vif Binding

Markus-Frederik Bohn[*,1], Shivender M. D. Shandilya[*,1], Mohan Somasundaran[1], Celia A. Schiffer[1]

[1]Department of Biochemistry and Molecular Pharmacology, [2]Department of Pediatrics and Program in Molecular Medicine, University of Massachusetts Medical School Worcester, Massachusetts, USA

The crystal structure of the catalytic, Vif-interacting domain of APOBEC3F is presented here. This structure reveals residues previously described as critical for Vif-dependent degradation of APOBEC3F to be located on a predominantly negatively charged contiguous surface. Sequence motifs forming an asymmetric crystallographic interface, between otherwise identical domains, influence dynamic equilibria of *in-vitro* oligomerization states and are conserved between APOBEC3F and HIV-1 Vif. While these regions have not been directly implicated in Vif binding to APOBEC3F, this example of molecular mimicry could play a role in modulating the solution state of the protein or other molecular recognition events. Structural comparisons with APOBEC3G, which binds HIV-Vif via the non-catalytic domain, suggest potentially different modes of binding in each enzyme. These structures will provide a scaffold to elucidate the APOBEC3 polyubiquitination complex and provide insights into oligomerization dynamics that will establish APOBEC3F as a structural target for novel antiretroviral strategies.

1. Dynamic Analysis of HIV-1 Gp120 Glycoprotein and Its Implications to Trimer Model Design

Tuoling Qiu[1] and David F. Green[1,2]

[1]Chemistry Department, Stony Brook University, Stony Brook, NY 11794; [2]Department of Applied Mathematics and Statistics, Stony Brook University, Stony Brook, NY 11794

HIV-1 envelope glycoprotein gp120 subunits, together with transmembrane glycoprotein gp41, form trimeric spikes on the viral surface. These spikes play an important role in initiating HIV-1 infection by binding of gp120 to CD4 receptors and subsequently to coreceptors (CCR5 or CXCR4) on target cells. An atomic detail model of the structure and dynamics of full-length, glycosylated gp120 in its native state remains elusive. Our ultimate goal is thus to build such a model and elucidate dynamic variations of gp120 upon binding. Firstly, to fully explore the conformational space of gp120 as a monomer, we have performed three independent long-time (1 ms +) molecular dynamics (MD) simulations for the unglycosylated monomeric gp120 core in its free, CD4-bound, and antibody-bound states. Through calculating pairwise RMSDs, RMS fluctuations and inter-domain distances, we have identified flexible and stable regions of gp120 core consistent with experimental results. Molecular motions of three subdomains of gp120 in three states are described in detail and characteristic configurational ensembles are derived by clustering analysis. Comparing secondary structure evolution and energetic decomposition data between different trajectories, regions critical to binding and local conformational changes during the simulations are revealed. Furthermore, we present initial results on characterization of the effects of variable loops and glycosylation on gp120 structure and dynamics.

2. Functional Characterization and Oligomeric State of Real Like Full Length gp41 Ectodomain

Koyeli Banerjee, David P. Weliky

Department of Chemistry, Michigan State University, East Lansing, MI 48824

Acquired Immune Deficiency Syndrome (AIDS) is a disease of the human immune system caused by the Human Immunodeficiency Virus (HIV). According to World Health Organization, globally there were 33 million people living with HIV till the year 2009. Every year ~2.6 million new cases of HIV infection are reported with 1.8 million annual deaths worldwide[1]. HIV infects the host cell in a two step process. It involves (1) fusion of the viral membrane with the host cell membrane and (2) release of the viral genetic material into the host cell which eventually integrates with the host genome. The entire process is mediated by a membrane glycoprotein in HIV called gp160. gp160 is comprised of an outer receptor binding subunit, gp120 and an inner fusion protein, gp41[2]. The membrane spanning gp41 participates in the fusion of the viral membrane with the host cell membrane and helps the release of viral genetic material into the host cell. The gp41 consists of different regions such as N-terminal fusion peptide (FP), an N-terminal heptad region (NHR), a loop region, a C-terminal heptad region (CHR), membrane proximal ectodomain region (MPER), the transmembrane region (TM), and the endodomain (cytoplasmic tail).

X-ray crystallographic structure of NHR-Loop-CHR region shows trimeric form[3]. Extensive biophysical characterization of gp41 ectodomain including the MPER has already been done in which the significance of the MPER has been shown[4, 5, 6, 7]. Broadly neutralizing antibodies are capable of blocking different strains of virus from infecting target cells. 2F5 and 4E10 are the two broadly neutralizing antibodies that bind to the MPER only in the PHI state[7].

However, all the constructs could be solubilized and stabilized only by attaching larger protein/peptide tags. We are successful in designing of the real like whole ectodomain region of gp41 without any tag. Discussion will be done on the functional characterization and oligomerization of the gp41 ectodomain. This work would take a step forward towards the improvement of immunogenicity of the vaccine and thus towards vaccine development.

References:

1. http://www.who.int/mediacentre/news/releases/2009/en
2. White M. W., Delos S. E., Brecher M., Schornberg K., *Critical Reviews in Biochemistry and Molecular Biology*, 2008, 43, 189-219
3. Weissenhorn W., Dessen A., Harrison S.C., Skehel J. J., Wiley D. C., *Nature*, 1997, 387,426-430
4. Liu J., Deng Y., Dey A. K., Moore J. P., Lu M., *Biochemistry*, 2009, 48, 2915-2923
5. Shi W., Bohon J., Han D. P., Habte H., Qin Y., Cho M. W., Chance M. R., *J. Biol. Chem.*, 2010, 285, 24290-24298
6. Gao G., Wieczorek L., Peachman K. K., Polonis V. R., Alving C. R., Rao M., Rao V. B., *J. Biol. Chem.*, 2013, 288, 234-246
7. Frey G., Peng H., Rits-Volloch S., Morelli M., Cheng Y., Chen B., *Proc. Natl. Acad. Sci. USA*, 2008, 105, 3739-3744

3. Interactions of Peptide Triazole Thiols with Env gp120 Induce Irreversible Breakdown and Inactivation of HIV-1 Virions

Arangassery Rosemary Bastian[1,2], Mark Contarino[1], Kantharaju Kamanna[1], Diogo Rodrigo deMagalhaes Moreira [1,3], Kevin Freedman[4], Karyn McFadden[5], Caitlin Duffy[1], Ali Emileh[6], Jeffrey Jacobson[7], James A. Hoxie[8], Irwin Chaiken[1]

Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA, 19102[1], Department of Pharmaceutical Sciences, Federal University of Pernambuco, Reficè-PE, Brazil,[3] Department of Mechanical Engineering and Mechanics, Drexel University, Philadelphia, PA, 19104,[4] Department of Molecular Genetics and Microbiology, Duke University, Durham, N.C., 27710,[5] Department of Chemical and Biological Engineering, Drexel University, Philadelphia, PA, 19104,[6] Department of Medicine, Drexel University College of Medicine, Philadelphia, PA, 19102,[7] Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, 19104.[8]

HIV-1 entry, mediated by the viral envelope glycoproteins gp120 and gp41, is an attractive target for preventing infection. Previously, we found that KR13, a sulfhydryl-containing peptide triazole, can bind to gp120, block CD4 and co-receptor binding, inhibit viral infectivity, and physically disrupt viral particles. Here, we investigated the mechanism of this effect. We found that KR13 and its parent non-sulfhydryl peptide, HNG156, induce gp120 shedding, although only KR13 induced release of the core protein p24. Viral lysis required KR13's free thiol, as blocking this group inhibited p24 release. The kinetics and products of virion disruption were evaluated using gradient centrifugation, biochemical assays, and transmission electron microscopy. Viral inactivation and gp120 shedding had a similar time-dependence, while p24 release lagged temporally. Although gp41 remained associated with particles after gp120 shedding and p24 release, virions lacked an organized capsid. The gp41 on the residual peptide treated HIV-1 virions were immunologically active. These were recognized by two conformationally dependent, neutralizing antibodies, 2F5 and 4E10. Interestingly, viral lysis was completely inhibited by T20, which blocks formation of the gp41 6-helix bundle during membrane fusion. Collectively, these findings indicate that KR13 peptide triazole initially causes viral inactivation through the release of gp120 followed by subsequent interactions with its free sulfhydryl leading to 6-helix bundle formation, viral membrane disruption, and p24 release. Our data are consistent with a model in which this peptide triggers structural changes in the HIV-1 trimer typically associated with viral entry and membrane fusion, and that in the absence of target cells, these changes result in irreversible viral inactivation and lysis. The potent and specific activity of this novel compound and its ability to inactivate virions prior to target cell engagement suggest that KR13 could be highly effective as a microbicide in inhibiting HIV transmission across mucosal barriers as well as a probe to understand biochemical signals required for membrane fusion.

4. Effects of the Env Membrane-Protein Interface in the Lytic Inactivation of HIV-1 by Peptide Triazole Thiols

Ramalingam Venkat Kalyana Sundaram[1,2], Cameron Abrams[3], and Irwin Chaiken[2]

[1]School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA 19104; [2]Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA 19102; [3]Chemical and Biological Engineering, Drexel University, Philadelphia, PA 19104

We are investigating the interplay between Env membrane and membrane-associated protein components in the virolytic inactivation of HIV-1 by peptide triazole thiols. The peptide triazole class of HIV-1 Env inhibitors targets gp120, inhibits virus–cell infection and causes gp120 shedding. Recent work has shown that peptide triazoles containing a free thiol, including the prototype KR-13, also cause leakage of core protein from within the virus lumen. Both membrane and membrane-associated protein components could play a role in the lytic process. The main lipid of the viral envelope is cholesterol (45-mol %). When treated with the cholesterol-sequestering methyl β-cyclodextrin (MβCD), a striking two-stage effect was observed on p24 capsid protein leakage induced by KR-13. At low concentrations of MβCD, p24 release increased in magnitude with MβCD dose. In contrast, high doses of MβCD, presumably from further extraction of cholesterol, caused a sharp desensitization resulting in a drop in KR-13 – induced p24 leakage. Virus treatment with sphingomyelinase, which converts sphingomyelin to ceramide, showed a similar 2-stage p24 release. Since sphingomyelin is known to associate with membrane cholesterol, the effects of cholesterol and sphingomyelin depletion may be related. Upon cholesterol-depletion of viruses in the absence of KR-13, a similar 2-stage effect was observed on virus cell infectivity. It was not observed when using an alternate Env pseudotyped virus that undergoes infection by an alternate mechanism, (Vesicular Stomatitis Virus G, VSV-G). The data obtained suggest that (1) the 2-stage effect due to cholesterol extraction via MβCD is specific to HIV-1 Env and its membrane opening processes; (2) similar mechanisms within the membrane of HIV-1 affect both infectivity of the virus and virus lysis caused by peptide triazole thiols; (3) membrane lipid components play a role in KR-13 induced spike protein effects leading to virus lysis. To understand the individual roles of the players in the lysis process, evaluation of effects from lipid modification are being carried out coordinately with efforts to evaluate the roles of membrane-associated Env protein components, such as gp41 CRAC, TM and C-terminus domains, on the peptide triazole lysis function. Understanding the virolysis mechanism will help define fundamental aspects of the virus-cell membrane interface of HIV-1 and assess approaches for virus inactivation by specific membrane disruption using Env targeting.

5. Membrane Structure Correlates to Function of LLP2 on the Cytoplasmic Tail of HIV-1 gp41 Protein

Alexander L. Boscia[1], Kiyotaka Akabori[1], Zachary Benamram[1], Jonathan A. Michel[1], Michael S. Jablin[1], Jonathan D. Steckbeck[2,3], Ronald C. Montelaro[2,3], John F. Nagle[1] and Stephanie Tristram-Nagle[1]

[1]Biological Physics Group, Physics Department, Carnegie Mellon University, Pittsburgh, Pennsylvania, USA; [2]Center for Vaccine Research and [3]Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

Mutation studies previously showed that the lentivirus lytic peptide (LLP2) sequence of the cytoplasmic C-terminal tail (CTT) of the HIV-1 gp41 envelope (Env) protein inhibited viral-initiated T-cell death and T-cell syncytium formation but did not affect virion infectivity. To examine the role of LLP2/membrane interactions, we applied synchrotron X-radiation to determine structure of hydrated membranes. We focused on WT LLP2 peptide (+3 charge) and MX2 mutant (-1 charge) with membrane mimics for the T-cell and the HIV-1 membranes. In order to investigate the influence of electrostatics, cholesterol content and peptide palmitoylation, we also studied three other LLP2 variants and HIV-1 mimics without negatively charged lipids or cholesterol as well as extracted HIV-1 lipids. All LLP2 peptides bound strongly to T-cell membrane mimics, as indicated by changes in membrane structure and bending. In contrast, none of the weakly bound LLP2 variants changed the HIV-1 membrane mimic structure or properties. This correlates well with, and provides a biophysical basis for, previously published results that reported lack of a mutant effect in the HIV virion and an inhibitory effect in T-cell syncytium formation.

6. Towards Understanding the Molecular Determinants of Peptide Triazole Interactions with HIV-1 Env gp120

Rachna Aneja[1], Andrew Holmes[1], Emily Arturo[1], Caitlin Duffy[1], Huiyuan Li[1], Ali Emileh[1,3], Judith Lalonde[2], Cameron Abrams[3], Irwin Chaiken[1]

Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA 19102; [2]Chemistry, Bryn Mawr College, Bryn Mawr, PA 19010; [3]Chemical and Biological Engineering, Drexel University, PA 19104

The HIV-1 gp120 envelope glycoprotein remains a compelling and yet elusive target for preventive and therapeutic interventions against the virus. Peptide triazoles (PT) are a novel class of molecules that bind to and trigger shedding of gp120 from the HIV-1 virion, thereby irreversibly inactivating the virus. Moreover, PT variants that contain an additional free C-terminal SH group cause HIV-1 specific lysis. Determining the structural mechanism of PT interactions is being pursued to help define the mode of action of this class of virus inactivators and also to facilitate design of structure-minimized PT inhibitory molecules with improved stabilities and antagonistic functions. Site-specific mutagenesis and all-atom explicit solvent molecular dynamics (MD) have been employed to investigate the functional site for peptide triazole inhibition on Env gp120. These analyses have identified binding pockets in gp120 for functionally important structural elements in PT's, and further have shown the capacity of PT's to stabilize an open conformation of gp120 containing an unstructured bridging sheet domain. Within the gp120 binding epitope, the functionally-critical residues D474 and T257 overlap the CD4 Phe 43 binding site of gp120. These residues also were found to be important for PT inhibition in pseudovirus Env gp120 mutagenic analyses. Results from ongoing molecular-level structure-activity experiments using both peptide and gp120 variants indicate that the PT's highly important Trp side chain contributes to the stability of the PT−gp120 complex through interactions with residues T257 and S375. We have determined the binding characteristics of T257 mutants with PT coordinately modified in the Trp position. The results suggest the importance of Trp indole nitrogen - T257 interaction in maintaining PT binding to gp120.

Current studies also include evaluation of truncated PT's (such as UM15, INNIXW, where X=ferrocenyltriazolePro) and core gp120's as backgrounds for structure-function analysis and high-resolution structure determination.

7. Mode of Action of the Sulfhydryl Group in Virolytic Peptide Triazole Thiol Inhibitors of HIV-1 Env

Lauren D. Bailey, Caitlin Duffy, Rachna Aneja, Rosemary Bastian, Huiyuan Li, Ali Emileh and Irwin Chaiken

Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA, 19102.

We have identified a class of peptide triazole thiol inhibitors that bind Env gp120, allosterically inhibit CD4 receptor and co-receptor surrogate mAb 17b binding, and cause both gp120 shedding and p24 release from the virus lumen. The prototype peptide triazole thiol KR-13 (R-I-N-N-I-X-W-S-E-A-M-M-βA-Q-βA-C-CONH2, X= ferrocenyltriazole-Pro) had an EC50 = 25 ± 6 nM for inhibition of virus cell infection and EC50 = 866 ± 55 nM for p24 release. In contrast, KR-13b (C-terminal SH capped by acetomidomethyl) inhibited cell infection but did not cause p24 leakage. We are investigating the mode of action by which the C-terminal sulfhydryl group causes irreversible inactivation. A working model is that the thiol interferes with conserved disulfides clustered proximal to the CD4 binding site in gp120 through “disulfide exchange”, leading to a rearrangement of the Env protein spike and ultimately disrupting the viral membrane. These disulfides are the 296-331 (V3 loop) disulfide, the 385-418 (C4) disulfide, the 378-445 (C3) disulfide, and the 119-205 disulfide (V1/V2 loop). From a molecular dynamics simulated model of the peptide triazole-gp120 complex, we estimate that the Trp residue of the IXW pharmacophore of KR13 ranges from 12 to 33 Å away from these disulfides. To evaluate the importance of the spatial relationship between PT-SH and gp120 disulfide groups, we have synthesized truncated and extended peptides derived from KR-13. From a comparison of p24 release and antiviral activity, we have observed a strong dependence of lysis activity on length of the linker between the IXW pharmacophore and SH group, an observation consistent with the disulfide exchange model of PT- SH action. The results suggest that there is a minimum length needed to contact disulfides of the envelope protein. We currently plan to refine the spacial and linker requirements of the SH group from the central peptide triazole binding the IXW pharmacophore, and to investigate the importance of disulfide exchange in the virolytic process as well as the specific Env disulfides that may be involved.

8. Multiple Receptor Protocol for DOCK to Target gp41

Brian C. Fochtman[1] and Robert C. Rizzo[2,3,4]

[1]Department of Biochemistry and Cellular Biology, [2]Department of Applied Mathematics and Statistics, [3]Institute of Chemical Biology & Drug Discovery, [4]Laufer Center for Physical and Quantitative Biology Stony Brook University, Stony Brook, NY 11794

Identification of lead compounds during a virtual screening applications may be limited by our ability to consider multiple conformations of the target protein. Here we discuss two algorithms, termed Multiple Average Receptor (MAR) and Multiple Independent Receptor (MIR), implemented into the program DOCK that sample the conformational space of the target during computational screening efforts. These algorithms rely on a grid based model of user defined conformers to improve computational tractability relative to explicit sampling and are being validated through pose-reproduction and enrichment studies employing the SB2010 and DUD-E databases. Conformers have been selected from MD simulation 'snapshots' and from multiple crystal structures. Results will be presented comparing these approaches versus standard single grid techniques for HIV RT, HIVPR, and other targets. Efforts using a *de novo* approach to automate sampling of 'R' groups on known-active scaffolds of gp41 inhibitors previously identified by our group (Holden et al. *Bioorg Med Chem Lett* **2012**, *22*, 3011-3016) will also be discussed.

9. Computational Protein Design of Carbohydrate Binding Proteins

Yiwei Cao[1,2] and David F. Green[1,2]

[1]Department of Applied Mathematics and Statistics, Stony Brook University, Stony Brook, NY, 11794-3600; [2]Laufer Center for Physical and Quantitative Biology, Stony Brook University, Stony Brook, NY

Protein-carbohydrate interactions play a crucial role in a variety of biological processes such as infection, immune response and signal transduction. A detailed understanding of protein-carbohydrate interactions would enable us to develop robust computational methods to discover variants with desired structural and functional properties. As protein-carbohydrate systems have not been extensively considered in a design context, our goal is to develop a methodology for design of carbohydrate binding proteins with high affinity to specific glycan targets. We have selected *Microcystis viridis* lectin (MVL) for our model system. MVL is a homodimer with each monomer containing two pseudo-symmetric domains; each domain contains a carbohydrate binding site which specifically binds the core structure of N-linked oligosaccharides. It has been shown to be an antiviral agent that inhibits HIV viral-cell fusion at nanomolar concentration through high-affinity interactions with the envelope glycoprotein gp120. Beginning with an ensemble of structures extracted from molecular dynamic simulation trajectories, we utilized an iterative protocol consisting of fixed-backbone design, backbone relaxation and subsequent redesign of low-energy sequences. In addition, we explored the influence of allowing flexibility of carbohydrate by incorporating its structural freedom into the search space. Our initial results suggest a number of interesting approaches to stabilizing MVL-carbohydrate complexes.

10. Discovery of CXCR4/CCR2 Dual-Targeted Fusion Inhibitors by Structure-Based Drug Design

Jie Xia[1, 2], Hongwei Jin[1], Zhenming Liu[1], Liangren Zhang[1]*, Xiang Simon Wang[2]*

[1]State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100191; [2] Molecular Modeling and Drug Discovery Core for District of Columbia Developmental Center for AIDS Research (DC D-CFAR); Laboratory of Cheminfomatics and Drug Design, Department of Pharmaceutical Sciences, College of Pharmacy, Howard University, Washington, DC 20059

Human CCR2 and CXCR4 receptors are members of Rhodopsin-like G Protein-Coupled Receptors (GPCRs) and associated with various inflammatory and infectious diseases. Since 1990s, CXCR4 has been discovered as one of co-receptors that are required for cellular entry by the human immunodeficiency virus (HIV-1) thus remains to be a major anti-HIV target. Although lots of efforts have been made to discover CXCR4 antagonists, the recent failure of AMD3100 in clinical trials indicates that the blockade of the single target such as CXCR4 appears to be insufficient to reach clinical efficacy and safety. Recently, polypharmacology studies of human chemokine receptors showed that CCR2 and CXCR4 co-exist in the form of hetero-oligomeric complex at the surface of T cells and monocytes. The specific antagonists of one receptor lead to functional cross-inhibition of the others. Therefore, CXCR4/CCR2 dual-targeted antagonists are expected to lead to reinforced inhibition against CXCR4 receptor. In this study, we designed the workflow for rationally designing CXCR4/CCR2 dual-targeted fusion inhibitors into two parts: building CCR2 comparative models and virtual screening based on CCR2 and CXCR4 3D structures. In the first part, we used Modeler 9v4 to build crude models for CCR2 and then customized two different ligand-steered homology modeling (LSHM) protocols to optimize the crude models, i.e.: Discovery Studio 2.5.5/Ligand Minimization and EN-NMA+ flexible docking by AutoDock Vina. After that, retrospective small-scale virtual screening was conducted, which was based on CCR2-specific ligand/decoy set generated by our novel and versatile protocol to build target-specific benchmarking sets. Finally, the best CCR2 model was selected out according to small-scale virtual screening and mutagenesis data for CCR2 binding. In the second part, we screened TimTec diversity set by molecular docking with the best CCR2 homology model and CXCR4 crystal structure. Results show that 1) the benchmarking set generated by our methodology is of good quality: ROC AUCs are close to 0.5 and ROC curves are in proximity to random distribution. 2) The best CCR2 model differentiates actives and inactives and enriches ligands largely with EF_1=17.24, EF_2=10.34, EF_5= 7.59, EF_{10}= 5.17, which is from the protocol of EN-NMA+ flexible docking by AutoDock Vina. Meanwhile, it shows a reasonable binding mode, which is consistent with the published site-directed mutagenesis data. 3) Based on this optimized CCR2 model and CXCR4 crystal structure, we obtained 10 potential dual-targeted hits. Those hits are now validated for their *in vitro* and *in vivo* activities by NIMH PDSP. In a conclusion, EN-NMA + flexible docking by AutoDock Vina as a protocol of LSHM performs better than Discovery Studio 2.5.5/Ligand Minimization whose optimization ability is seemingly limited. Furthermore, the scoring function called PMF04 is particularly suitable for CCR2-based drug discovery. The bioassay validation of the dual-targeted hits is currently undergoing.

11. Use of Molecular Mimicry in the Design of HIV Fusion Inhibitors

William J. Allen[1] and Robert C. Rizzo[1,2,3]

[1]Department of Applied Mathematics and Statistics, [2]Institute of Chemical Biology & Drug Discovery, [3]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, New York, 11794

HIV-cell fusion is mediated by the viral glycoprotein 41 (gp41), an attractive target for therapeutic intervention due to the transient exposure of conserved regions during the fusion process. The only currently approved drug targeting gp41 is a 36-amino acid peptide (T20, Fuzeon) that gives rise to drug-resistant variants of HIV. We have recently implemented a *de novo* design algorithm into the virtual screening program DOCK6 wherein new small molecule inhibitors can be designed to mimic specific protein-protein interactions that are integral to the HIV fusion mechanism, potentially avoiding resistance mutations. Using this method, fragments of pre-existing molecules were assembled in the context of the conserved, hydrophobic pocket on the surface of the gp41 N-heptad repeat trimer. Fragment assembly was guided by the DOCK6 molecular mechanics-based interaction energy function in combination with a "footprint" similarity function, which exploits knowledge of specific binding interfaces. Populations of candidate molecules were also constrained by chemical properties including molecular weight, number of rotatable bonds, and other Lipinski-type descriptors. New molecules assembled in this study exhibited favorable predicted binding energies and specifically satisfied desired conserved interactions. Other putative small-molecule binding pockets, including two at the interface of the N-heptad repeats, are being explored. In addition, several previously-identified lead compounds with known gp41 activity are being optimized by these methods. Plans for experimental follow-up are in development.

12. gp41 Ectodomain Constructs for Small Molecule-Protein Complexes

Joseph D. Walsh[1,2], Shidong Chu[1], Vladimir Sofiyev[1], Guangyan Zhou[1], Hardeep Kaur[1], Miriam Gochin[1,2]

[1]Department of Basic Science, Touro University. 1310 Club Dr., Vallejo CA 94594; [2]Department of Pharmaceutical Chemistry, UCSF, San Francisco CA 94158.

Structural studies of ligand interactions with the HIV gp41 ectodomain require the production of samples with ligands bound in shallow hydrophobic pockets on the gp41 surface. Because gp41 is a homo-trimer this results in significant exposure of hydrophobic surface area of the protein, making expression, purification, and stabilization of the protein challenging. In order to address this problem, we have devised gp41 constructs wherein the hydrophobic pocket is covered during over-expression in *E. coli*, leading to large amount of soluble, ~95% pure protein after the first purification step (up to 100mg/L of minimal media). This precursor protein is then cleaved, exposing a varying amount of hydrophobic surface, depending on the construct. In order to further stabilize the gp41 construct, we have also devised a class of very soluble “Cover Proteins” which bind weakly to the exposed hydrophobic surface on the gp41 construct. In this way we are able to screen the gp41 construct against aggregation, even at elevated temperatures. We are in the process of using this approach to produce ligand-protein complexes using novel inhibitors identified in this laboratory.

13. Targeting HIV gp41 Fusion: Binding Affinity Characterization using Thermodynamic Integration and Pharmacophore-based Scoring in DOCK6

Lingling Jiang[1] and Robert C. Rizzo[1,2,3]

[1]Department of Applied Mathematics & Statistics, [2]Institute of Chemical Biology & Drug Discovery, [3]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, NY 11794

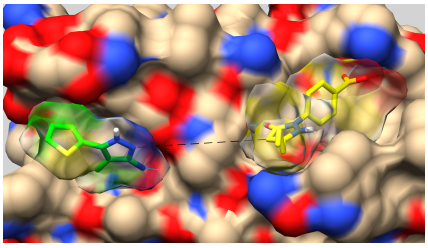
Primary resistant mutations that occur in the gp41 N-terminal heptad repeat region, as well as compensatory mutations in the C-terminal domain, are of great interest for designing next generation HIV fusion inhibitors. In this study, atomic-level molecular dynamics (MD) simulations employing free energy calculation protocols were performed to computationally evaluate the changes in binding affinity introduced by single point mutations S138X (X=20 a.a) on the peptide drug T20 while bound to three gp41 variants (wild-type, N43D, and V38A). Calculated relative binding energy for twenty representative complexes predicted by the Thermodynamic Integration (TI) method show reasonable agreement with experiment (r^2=0.66). Per-residue component energy analyses used to uncover the determinant factors driving differential binding for T20 with gp41 will also be presented. Using the resulting peptide binding profiles as a reference, a 3D pharmacophore matching similarity (FMS) based scoring function is being developed for DOCK6 to guide small molecule lead discovery. Preliminary validations indicate reasonable performance in pose identification success rate tests using our large docking database SB2010.

14. Fragment Screening Targeting HIV-1 Fusion Glycoprotein-41

Shidong Chu, Miriam Gochin

Touro University California, Vallejo, CA 94592

The HIV-1 envelope glycoprotein gp41 fusion intermediate is a promising drug target for inhibiting viral entry. However, drug development has been impeded by challenges inherent in mediating the underlying protein-protein interaction. Here we report on the identification of fragments that bind to a C-terminal sub-pocket adjacent to the well-known hydrophobic pocket on the NHR coiled coil. Using a specifically designed assay and ligand-based NMR screening of a fragment library, we identified a thiophenylaminopyrazole compound with a dissociation constant of ~500μM. Interaction with the C-terminal sub-pocket was confirmed by paramagnetic relaxation enhancement NMR experiments, which also yielded the binding mode. Shape-based similarity searching detected additional phenylpyrazole and phenyltriazole fragments within the library, enriching the hit rate over random screening, and revealing molecular features required for activity. Discovery of the novel scaffolds and binding mechanism suggests avenues for extending the interaction surface and improving the potency of a hydrophobic pocket binding inhibitor.



15. Property-Based Volume Overlap as a DOCK Scoring Function

Yuchen Zhou[1] and Robert C. Rizzo[1,2,3]

[1]Department of Applied Mathematics and Statistics, [2]Institute of Chemical Biology & Drug Discovery, [3]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, NY 11794

Scoring functions are an essential component for accurate virtual screening and *de novo* design of lead compounds. Here, we present a new volume overlap scoring function recently implemented into the program DOCK that describes the conformational and chemical similarity of two molecules by determining the extent of volume overlap in a binding pocket site. Work in progress will be presented in which we performed a series of pose identification, enrichment and cross docking tests. Preliminary results suggest a property-based volume overlap similarity score yields improved predictive ability compared to the standard DOCK score. Application of volume overlap similarity with *de novo* design, currently under development in our lab to target HIVgp41, will also be discussed.

16. Structural and Functional Characterization of Viral Fusion Peptides by Lipid Mixing Assays and Solid-State NMR

Li Xie, Ujjayini Ghosh, David Weliky

Department of Chemistry, Michigan State University, East Lansing, MI 48824

HIV fusion peptide (HFP) and influenza fusion peptide (IFP) both play an important role in the host cell-virus membrane fusion process. The viral fusion peptides HFP and IFP are located at the N-terminus of the HIV gp41 fusion protein and influenza hemagglutinin fusion protein (HA2), respectively.[1] In this work, lipid mixing assays were performed to study the fusion activities of both wild-type and mutant HFP constructs. Figure 1 displays lipid mixing assay results of different HFP constructs, where wild-type HFP shows the highest fusion activity, followed by V2E, L9R, and L9E mutants. The difference in fusion activity of wild-type and mutant HFP might be due to their different membrane insertion depths.[2] ^{13}C-^{2}H rotational-echo double resonance (REDOR) solid-state NMR experiments were performed to detect the membrane insertion of HFP. REDOR data indicate there might be a membrane location distribution of HFP in lipid membrane.[3] In this work, we also studied the structure of membrane-associated IFP by ^{13}C-^{15}N REDOR. The data support a ~ 60% population of semi-closed structure and a ~40% population of closed structure of the membrane-associated IFP at pH 5.0.[4]

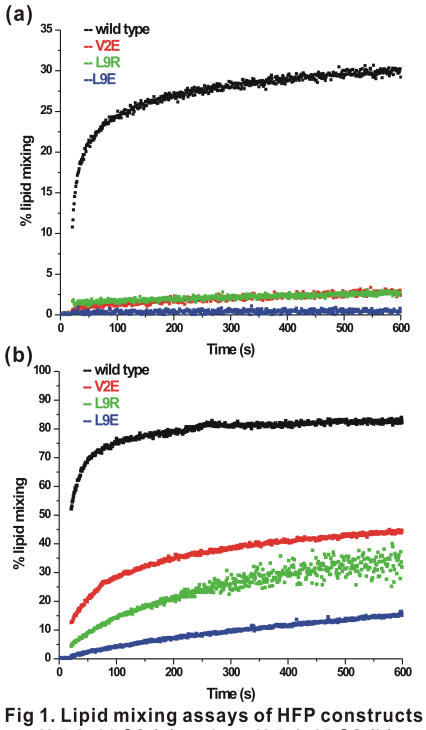


Fig 1. Lipid mixing assays of HFP constructs at pH 7.8, 30 °C (a) and at pH 7.4, 37 °C (b). HFP:POPC:POPG:Cholesterol=1:40:10:25.

References:

1. White, J., et al. Critical Reviews in Biochemistry and Molecular Biology **2008**, 43, 189-219.
2. Qiang, W., et al. Proceedings of the National Academy of Sciences of the United States of America **2009**, 106, 15314-15319.
3. Xie, L., et al. In preparation.
4. Xie, L., et al. Journal of Biomolecular NMR **2013**, 55, 139-146.

17. Structure of Cyclophilin A in Complex with HIV-1 Capsid Assembly by CryoEM

Chuang Liu and Peijun Zhang

Department of Structural Biology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15260, USA

The cellular protein cyclophilin A (CypA) plays an important role in HIV-1 replication through interaction with the core. CypA binds specifically to the human HIV-1 CA protein and enhances the replication of many isolates of HIV-1. Blocking CypA binding by competitive binding of CypA with the immunosuppressive drug cyclosporin A (CsA), or via mutations in CA, reduces HIV-1 infectivity. It has been proposed that CypA exerts its function in the early phase of viral entry before reverse transcription, perhaps through uncoating, but the molecular mechanism of CypA in promoting HIV-1 infection has not been defined to date. To understand the detailed molecular interactions between HIV-1 capsid and CypA, and structural and functional effects of CypA on uncoating, we determined the structure of HIV-CA/CypA complex assemblies to 8 Å resolution using cryoEM. The resulting map displays very well-ordered CA hexamer helical lattice, resolving all the α-helices within the CA molecule, but less well-ordered CypA density, indicating that CypA binding is flexible. The CypA density appears bridging two neighboring CA hexamers on the outer surface of the tubes, directly above the CA-CTD dimer interface. More intriguingly, the CypA density linking the neighboring CA hexamers occurs predominately along the most curved, short-pitched helical array. In contrast, little CypA density was observed along the helical array parallel to the tube axis. Furthermore, aligning the CA-NTD in the crystal structure of CypA/CA-NTD complex (1AK4) with the MDFF fitted CA-hexamers places the CypA molecular right into this bridging density, resulting in two possibly interacting CypA molecules across two adjacent CA hexamers. It's not yet clear whether the two CypA molecules interact with each other and form a dimer, but one might speculate that the CypA bridge over two CA hexamers could be the site of action where CypA facilitates HIV-1 core uncoating.

18. Evidence for Biphasic Uncoating During HIV-1 Infection From a Novel Imaging Assay

Hongzhan Xu[1], Tamera Franks[1], Gregory Gibson[2], Kelly Huber[1], Christopher Aiken[3], Simon Watkins[2], Nicolas Sluis-Cremer[1], and Zandrea Ambrose[1]

[1]Division of Infectious Diseases, Department of Medicine and [2]Department of Cell Biology and Molecular Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA; [3]Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

Uncoating of the HIV-1 core plays a critical role during early post-fusion stages of infection but is poorly understood. Microscopy-based assays are unable to easily distinguish between intact and partially uncoated viral cores. In this study, we used 5-ethynyl uridine (EU) to label viral-associated RNA during HIV production. At early time points after infection with EU-labeled virions, the viral-associated RNA was stained with an EU-specific dye and was detected by confocal microscopy together with viral proteins. We observed that detection of the viral-associated RNA was specific for EU-labeled virions, was detected only after viral fusion with target cells, and occurred after an initial opening of the core. In vitro staining of cores showed that the opening of the core allowed the small molecule dye, but not RNase A or antibodies, inside. Also, staining of the viral-associated RNA, which is co-localized with nucleocapsid, decays over time after viral infection. The decay rate of RNA staining is dependent on capsid stability, which was altered by CA mutations or a small molecule inducer of HIV-1 uncoating. While the staining of EU-labeled RNA was not affected by inhibition of reverse transcription, the kinetics of core opening of different CA mutants correlated with initiation of reverse transcription. Analysis of the E45A CA mutant suggests that initial core opening is independent of complete capsid disassembly. Taken together, our results establish a novel RNA accessibility-based assay that detects an early event in HIV-1 uncoating and can be used to further define this process.

19. Tetramerization of SAMHD1 Is Required for Biological Activity and Inhibition of HIV Infection

Junpeng Yan[1], Sarabpreet Kaur[1], Maria DeLucia[2,3], Caili Hao[1], Jennifer Mehrens[2,3], Chuanping Wang[1], Marcin Golczak[4], Krzysztof Palczewski[4], Angela M. Gronenborn[2,3], Jinwoo Ahn[1,2,3,*], and Jacek Skowronski[1,2,3,*]

[1]Departments of Molecular Biology and Microbiology and [4]Pharmacology, Case Western Reserve School of Medicine, Cleveland, Ohio 44106; [2]Department of Structural Biology and [3]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15260

SAMHD1 is a dGTP-activated dNTPase that has been implicated as a modulator of the innate immune response. In monocytes and their differentiated derivatives, as well as in quiescent cells, SAMHD1 strongly inhibits HIV-1 infection and, to a lesser extent, HIV-2 and simian immunodeficiency virus (SIV) because of their virion-associated virulence factor Vpx, which directs SAMHD1 for proteasomal degradation. Here, we used a combination of biochemical and virologic approaches to gain insights into the functional organization of human SAMHD1. We found that the catalytically active recombinant dNTPase is a dGTP-induced tetramer. Chemical cross-linking studies revealed SAMHD1 tetramers in human monocytic cells, in which it strongly restricts HIV-1 infection. The propensity of SAMHD1 to maintain the tetrameric state in vitro is regulated by its C terminus, located outside of the catalytic domain. Accordingly, we show that the C terminus is required for the full ability of SAMHD1 to deplete dNTP pools and to inhibit HIV-1 infection in U937 monocytes. Interestingly, the human SAMHD1 C terminus contains a docking site for HIV-2/SIVmac Vpx and is known to have evolved under positive selection. This evidence indicates that Vpx targets a functionally important element in SAMHD1. Together, our findings imply that SAMHD1 tetramers are the biologically active form of this dNTPase and provide new insights into the functional organization of SAMHD1.

*Corresponding authors

20. HIV-2 and SIV_{mac} Accessory Virulence Factor Vpx Down-Regulates SAMHD1 Catalysis Independent of Proteasome-Dependent Degradation

Maria DeLucia[1,2,*], Jennifer Mehrens[1,2,*], Ying Wu[1,2], and Jinwoo Ahn[1,2]

[1]Department of Structural Biology and [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260; [*]These authors contributed equally.

SAMHD1, a dGTP-regulated dNTP triphosphohydrolase, down-regulates dNTP pools in terminally differentiated and quiescent cells, thereby inhibiting HIV-1 infection at the reverse transcription step. HIV-2 and SIV counteract this restriction via a virion-associated virulence accessory factor, Vpx (Vpr in some SIVs), which loads SAMHD1 onto CRL4-DCAF1 E3 ubiquitin ligase for poly-ubiquitination, programming it for proteasome-dependent degradation. However, the detailed molecular mechanisms of SAMHD1 recruitment to the E3 ligase, as mediated by divergent orthologous Vpx proteins encoded by HIV/SIV strains adapted to different primate species, have not been defined. We applied surface plasmon resonance analysis to assess the requirements for and kinetics of binding between various primate SAMHD1 proteins and Vpx proteins from SIV or HIV-2 strains. Our data indicate that Vpx proteins, bound to DCAF1, interface with the C-terminus of primate SAMHD1 proteins with nanomolar affinity, manifested by rapid association and slow dissociation. Further, we provide evidence that Vpx binding to SAMHD1 induces disassembly of dGTP-induced oligomer, which is the catalytically active form of the enzyme, thereby directly inhibiting its catalytic activity. Our studies reveal a previously unrecognized biochemical mechanism of Vpx-mediated viral countermeasure against an HIV restriction factor, SAMHD1: direct down-modulation of its catalytic activity, mediated by the same binding event that leads to SAMHD1 recruitment to the E3 ubiquitin ligase for proteasome-dependent degradation.

21. A Single Amino Acid in the Sterile Alpha Motif (SAM) Domain of Human SAMHD1 Alters Susceptibility to a Subset of Simian Immunodeficiency Virus (SIV) Virulence Factors Vpr and Vpx

Ying Wu[1,2], Jennifer Mehrens[1,2], Maria DeLucia[1,2], and Jinwoo Ahn[1,2]

[1]Department of Structural Biology and [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260

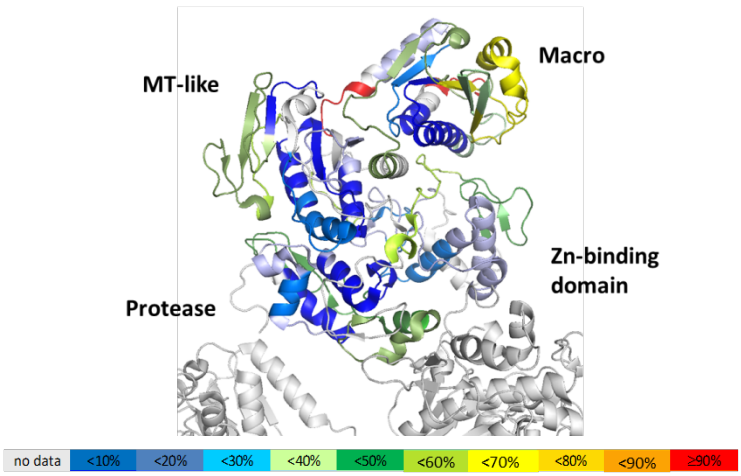
Sterile Alpha Motif (SAM) and Histidine/Aspartate (HD) containing protein 1 (SAMHD1) restricts HIV-1 infection in certain cell types. HIV-2 and SIV encode a virulence factor, termed Vpx (Vpr in some SIV), which counteracts SAMHD1 restriction. Current evidence suggests that the virulence factor recruits SAMHD1 to the Cullin4-Ring Finger E3 ubiquitin ligase (CRL4) by facilitating an interaction between SAMHD1 and a substrate receptor of the CRL4, DDB1- and Cullin4-associated factor 1 (DCAF1), for proteasome-dependent down-regulation. Evolutionary sequence divergence among Vpr and Vpx proteins has resulted in differential interaction interfaces between SAMHD1 and the viral proteins: two distinct regions of SAMHD1, namely the SAM domain and the C-terminus distal to the HD domain, are targeted independently by distinct Vpx (or Vpr) proteins, while, at the same time, an interaction with the C-terminal WD40 domain of DCAF1 is common among the viral proteins. Here, we use systematic biochemical and biophysical approaches, in combination with mutagenesis, to identify Ser52 as a critical determinant of human SAMHD1's ability to escape down-regulation by a subset of SIV Vpr and Vpx proteins. In particular, mutating Ser52 of human SAMHD1 to Phe, which is found at position 52 in African Green Monkey (AGM) and Red-capped Monkey (RCM) SAMHD1, results in recruitment of the mutated protein to the CRL4-DCAF1 by both SIV Vpr from AGM and SIV Vpx from RCM. Our findings suggest that these two virulence factors bind a conserved surface at the SAM domain of monkey SAMHD1 proteins.

22. Analysis of the Conformational Dynamics of Viral Proteins by HDX

Devrishi Goswami[1], Samantha Yost[2], Steve Tuske[3], Edward Arnold[3], Joseph Marcotrigiano[2] and Patrick Griffin[1]

[1]The Scripps Research Institute; [2]Rutgers University - Center for Advanced Biotechnology and Medicine; [3]Rutgers University, Chemistry & Chemical Biology

Many human viral pathogens encode proteins that are cleaved by viral or host proteases. However, considering its prevalence in many virus families, comprehensive structural information about the precursor forms of these proteins is lacking. Recently, the atomic structure of the precursor polyprotein p23$^{pro\text{-}zbd}$ from the replication complex of the SIndbis alphavirus was solved by the Marcotrigiano group. To further these studies we applied hydrogen/deuterium exchange (HDX) to characterize the dynamics of the native state of p23$^{pro\text{-}zbd}$. Analysis of the conformational dynamics of p23$^{pro\text{-}zbd}$ revealed several regions of the protein that are highly ordered as well as regions that demonstrate a high degree of dynamic behavior with the macro domain being the most dynamic compared to the protease, MT-like and Zn-binding domains. The next steps are to extend these studies to the characterization of the intact polyprotein as well as some functionally relevant point mutants.



We are initiating HDX characterization of the p51-p66 heterodimer of the HIV reverse transcriptase. As this protein complex is an asymmetric heterodimer, it provides a technical challenge for HDX analysis. HDX relies on performing on-exchange with deuterium followed by proteolysis and then detection by mass spectrometry. Thus it is impossible to determine which subunit of the heterodimer (p51 and p66) that sequence identical peptides belong to and it is likely that these peptides, depending on the subunit of origin, will show differential HDX kinetics. One strategy to address this issue is to ^{15}N enrich one of the subunits of the heterodimer and the other subunit would be normal isotopic distribution. These two subunits would then be mixed. This strategy will allow unambiguous ID of peptides from each subunit. An alternative approach is to subunit specific biotinylate, mix, then trap one subunit prior to proteolysis. This methodology optimization is ongoing and results will be presented.

23. Small Angle X-Ray Scattering Analysis of the HIV-1 vRNA 5´ UTR Initiation Complex Reveals a tRNA-Like Fold

Christopher P. Jones, William Cantara, Erik Olson, and Karin Musier-Forsyth

Department of Chemistry and Biochemistry, Center for RNA Biology, and Center for Retroviral Research, Ohio State University, Columbus, OH 43210

The highly structured 5´ untranslated region (5´ UTR) of the HIV-1 genome contains motifs critical to vRNA transcription, primer annealing (primer binding site, PBS), and packaging. Interactions with numerous viral and cellular factors also occur in the 5´-UTR region. The PBS-containing stem-loop shares extensive complementarity with the 3´ half of human $tRNA^{Lys3}$, which is the primer for reverse transcription. We have shown that human lysyl-tRNA synthetase, which plays a key role in packaging the tRNA primer, specifically binds to a tRNA anticodon-like element (TLE) in the HIV-1 genome (Jones et al, (2013) *RNA,* 19: 219). The U-rich TLE mimics the anticodon loop of $tRNA^{Lys}$ and is located proximal to the PBS. Our results suggest that HIV-1 uses molecular mimicry of the anticodon of $tRNA^{Lys}$ to increase the efficiency of $tRNA^{Lys3}$ annealing to viral RNA. Although high-resolution three-dimensional structures have been reported for short segments of the genome, the tertiary structure of the PBS/TLE domain is unknown. We hypothesize that the genome may mimic the entire three-dimensional tRNA fold. To test this idea and to gain new insights into the structure of the 5´ UTR, we carried out small-angle X-ray scattering (SAXS) analyses combined with a molecular dynamics (MD)-based approach. We optimized folding conditions for ~100-nt domains derived from the HIV-1 5´ UTR including TAR/PolyA, PBS/TLE (alone and in the presence of annealed $tRNA^{Lys3}$-derived sequences), and Psi. As expected, TAR/PolyA forms an extended coaxially-stacked helical domain. The apo-PBS/TLE forms an extended structure that includes a tRNA-like L-shaped fold in good agreement with SAXS data obtained for $tRNA^{Lys3}$. In the presence of an annealed DNA 18-mer complementary to the PBS or $tRNA^{Lys3}$, the structure is oriented similarly with additional envelope density consistent with an annealed complex. Our SAXS/MD derived model together with the known crystal structure of HIV-1 reverse transcriptase in complex with a DNA/RNA duplex allowed us to build a model of the initiation complex. Our data support the presence of a tRNA-like fold that likely recruits lysyl-tRNA synthetase to the PBS/TLE region of the vRNA to facilitate tRNA annealing and initiation of reverse transcription.

24. Small Angle X-ray Scattering Studies of the HIV-1 Reverse Transcriptase Initiation Complex

William C. Ho[1], Steve Tuske[1], Matthew T. Miller[1], Joseph D. Bauman[1], Jennifer T. Miller[2], Stuart F.J. Le Grice[2], and Eddy Arnold[1]

[1]Center for Advanced Biotechnology and Medicine, Department of Chemistry and Biology, Rutgers University, Piscataway, New Jersey, USA. [2]HIV Drug Resistance Program, NCI-Frederick Cancer Research and Development Center, Frederick, Maryland, USA

A critical step in the transcription of the HIV-1 genome is the recruitment of the host $tRNA^{Lys,3}$ as a primer to a 18 nucleotide region on the viral genome known as the primer binding site (PBS). HIV-1 Reverse Transcriptase (RT) recognizes this $tRNA^{Lys,3}$/PBS complex and initiates transcription. Despite decades of intensive research, the structure of the HIV-1 initiation complex (RT/ $tRNA^{Lys,3}$/PBS) remains unknown. Such a structure would represent a valuable piece of unlocking the puzzle that is the HIV lifecycle. Small angle X-ray scattering (SAXS) was used to investigate the structure of the RT initiation complex. SAXS data of the RT/ $tRNA^{Lys,3}$/PBScomplex were collected at the CHESS F2 BioSAXS beamline(F2) and processed using the program RAW. Envelope reconstructions were calculated using the program DAMMIF and DAMAVER. When comparing the envelope of RT alone against the envelope of RT/ $tRNA^{Lys,3}$/PBS, extra density was observed that may be attributable to the ~57 nt portion of RNA that protrudes from the nucleic acid binding cleft of RT (Puglisi, et. al., JMB (2011) **410**, 863-874). To better position RT in SAXS envelope reconstructions, we utilized the Fab28 antibody, which binds to a specific area on the underside of RT. SAXS data were collected and an envelope reconstruction was calculated of the RT/Fab28 complex. The positions of the Fab28 and RT can be clearly observed; we are currently in the process of pursuing the RT/ $tRNA^{Lys,3}$/PBS/Fab28 complex via SAXS.

We are grateful to NIGMS for support of the HIVE P50 Center (GM103368; Program Director: Arthur Olson, The Scripps Research Institute).

25. 5,6-Dihydro-5-aza-2'-deoxycytidine Potentiates the Anti-HIV-1 Activity of Ribonucleotide Reductase Inhibitors

Jonathan M. Rawson, Richard H. Heineman, Lauren B. Beach, Jessica L. Martin, Erica K. Schnettler, Michael J. Dapp, Steven E. Patterson, and Louis M. Mansky

Institute for Molecular Virology, University of Minnesota, Minneapolis, MN 55455, USA

The nucleoside analog 5,6-dihydro-5-aza-2'-deoxycytidine (KP-1212) has been proposed as a first-in-class lethal mutagen of human immunodeficiency virus type-1 (HIV-1). While initial studies in cell culture appeared quite promising, Phase II clinical trials of a prodrug form (KP-1461) found that it could not significantly reduce plasma viral loads in HIV-1-infected individuals by itself. We tested whether the antiretroviral activity of KP-1212 could be enhanced using inhibitors of ribonucleotide reductase (RNRIs). While we observed that KP-1212 had a minimal effect on HIV-1 infectivity and mutant frequency in cell culture, KP-1212 potentiated the activity of several RNRIs, leading to significant reductions in HIV-1 infectivity and corresponding increases in the viral mutant frequency without cellular cytotoxicity. We found through sequencing that the addition of KP-1212 to one particular RNRI, resveratrol, led to a marked induction of G-to-C transversions, a mutation rarely seen in the absence of drug. This is the first demonstration of a weak anti-HIV-1 mutagen having the capability to potentiate the antiretroviral activity of RNRIs. These observations could enhance the potential for clinical translation of weak viral mutagens for the treatment of HIV-1 infection.

26. APOBEC3G Monomers and Oligomers Bind Single-Stranded DNA

Ming Li and Reuben S. Harris

Department of Biochemistry, Molecular Biology & Biophysics, Institute for Molecular Virology, Center for Genome Engineering, University of Minnesota, Minneapolis, Minnesota, USA 55455

APOBEC3G (A3G) is a single-stranded DNA cytosine deaminase that has the capacity to restrict HIV-1 and other retroelements. Although A3G can exist in multiple oligomeric states, the relevance of this phenomenon to binding and deaminating single-stranded DNA is unclear. Here, we utilized a recombinant A3G protein purified from human 293T cells to systematically analyze the binding and deaminase activity of A3G on SS-DNA oligos. We demonstrated that A3G can bind 9-nucleotide oligo in a EMSA assay and deaminase 5-nucleotied oligo in a in vitro deamination assay, those indicated A3G can process substrate SS-DNA efficiently through its C-terminal catalytic domain. We also found that A3G can bind SS-DNA as monomer and oligomers in vitro and exert deamination at low A3G/Substrate ratio at which A3G binds SS-DNA primarily as monomer. We concluded that A3G can process substrate SS-DNA highly efficiently and flexibly not closely dependent on its oligomerization form.

27. Novel APOBEC3G Properties Allow it to be an Efficient Mutator of Newly Synthesized HIV-1 DNA

Roni Nowarski[1, 2], Edan Kenig[1], Prabhu Ponnandy[1] and Moshe Kotler[1]

[1]Department of Pathology and the Lautenberg Center for General and Tumor Immunology, the Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel; [2]Present address: Department of Immunobiology, Yale University School of Medicine, New Haven, CT 06520, USA

APOBEC3G (A3G) restricts retroviruses, hepatitis B, and several DNA viruses by editing single strand DNA (ssDNA) replication intermediates. A3G is encapsidated into the newly assembled virus as a multimer through interaction with HIV-1 or 7SL RNA molecules and the nucleocapsid protein. Following reverse transcription in the new target cell, A3G deaminates viral cDNA cytosines to uracils. Here we demonstrate that inside HIV-1 virions, A3G can target the newly synthesized strong stop (ssDNA) reverse transcripts and deaminate two out of three CCC motifs, one of which resides at the extreme 3' C terminus. However, these C to U alterations do not appear to hamper first strand transfer. Following reverse transcription, the HIV-1 RNase H endonuclease digests the viral genome, leaving ssDNA gaps, which can be used as a substrate for A3G. By using electrophoretic mobility shift assay (EMSA) and highly sensitive deamination assays, we demonstrated that blocking ssDNA substrates by DNA or RNA duplexes at the 3'- or 5' ends, or both, does not hamper A3G tethering and catalysis of the gapped substrates. Even a gap of 3 nt (CCC) can be used as a target for A3G. In addition, we showed that free ssDNA termini are not required for binding A3G to its substrate. Our findings elucidate characteristics of A3G that allow deamination of the newly synthesized reverse transcripts concomitant with the stripping of viral RNA by the viral RNase H, making it an efficient mutator of HIV-1 DNA.

28. Mouse APOBEC3 Deaminase: Biochemical Properties and Possible Regulation by Phosphorylation

Smita Nair, Silvia Sanchez-Martinez and Alan Rein

Retrovirus Assembly Section, HIV Drug Resistance Program, National Cancer Institute, Frederick, MD-21702.

APOBEC3 (A3) proteins are a family of cytidine deaminases that interfere with retroviral replication. Humans encode 7 A3 proteins (hA3A through H), hA3G being the most studied and well characterized. Mice only encode one A3 protein (mA3). The deaminase active site domain contains the conserved His-X-Glu motif and the Pro-Cys-X-X-Cys zinc finger motif. A3 proteins have either one or two such domains; in the case of hA3B, hA3F, and hA3G, the C-terminal domain is catalytically active and the N-terminal domain is involved in encapsidation into the virions.

The present study aims to investigate the biochemical properties of mA3 deaminase purified from insect cells. Purified mA3 protein is enzymatically active and deaminates ssDNA substrates at TCC and TTC target sites. Studies with mA3 active site and zinc finger mutants revealed that the deaminase activity resides in the N-terminal domain while the C-terminal domain (zinc finger and arginine rich motifs) is involved in encapsidation; however, both domains are required for the antiviral activity against ΔVif-HIV-1. This implies that mA3 has a reversed domain arrangement compared to hA3s.

Human A3G is known to processively deaminate cytosines in a 3' to 5' direction in vitro; this intrinsic property has been suggested to generate the 5' to 3' mutational gradient in vivo. In contrast, we have found that mA3 exhibits 5' to 3' deamination polarity in vitro. Interestingly, even though mA3 has a reversed intrinsic polarity compared to hA3G, a 5' to 3' gradient of G to A mutations (like that with hA3G) is maintained while deaminating ΔVif-HIV-1 in vivo. These findings suggest that an in vivo mutational gradient may not be a consequence of the intrinsic polarity of the deaminase, but rather the length of time the 3' end of the negative strand gDNA remains single stranded during reverse transcription.

Recent studies have revealed that phosphorylation by protein kinase A (PKA) plays an important role in regulating the antiviral activity of hA3G and in suppressing its intrinsic DNA deaminase activity. We have explored the possibility of this kind of regulation in mA3. mA3 has putative sites for phosphorylation by PKA (T42), CKII (T49, S89, S204) and PKC (S9, S25, T49, S89, S128, S249, T306, S363, T366). These residues were mutated either to aspartate (mimicking phosphorylation) or alanine (abolishing phosphorylation). T42D and S128D mutant mA3s exhibited no deaminase activity in vitro whereas both T306D and S363D mutants failed to be encapsidated into ΔVif-HIV-1 virions. All these mutants were also compromised in their antiviral activity against ΔVif-HIV-1. These results raise the possibility that the antiviral properties of mA3 could be regulated by phosphorylation.

This work is supported by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, National Institutes of Health.

29. Time-Resolved NMR of DNA Cytosine Deamination by APOBEC3G

Stefan Harjes, William Solomon, Ming Li, Kuan-Ming Chen, Elena Harjes, Reuben S. Harris, and Hiroshi Matsuo

Department of Biochemistry, Molecular Biology, and Biophysics, Institute for Molecular Virology, University of Minnesota, Minneapolis, Minnesota, USA

We used time-resolved NMR to determine kinetic parameters for APOBEC3G C-terminal domain (ctd) deamination of a single-stranded DNA substrate containing a single 5´-CCC hotspot motif.[1] A3Gctd exhibited 45-fold preference for 5´-CCC substrate over 5´-CCU substrate, which explains why A3G displays almost no processivity within a 5'-CCC motif. In addition, A3Gctd's shortest substrate sequence was found to be a pentanucleotide containing 5´-CCC flanked on both sides by a single nucleotide. A3Gctd as well as full-length A3G showed peak deamination velocities at pH 5.5. We found that H216 is responsible for this pH dependence, suggesting that protonation of H216 could play a key role in substrate binding. Protonation of H216 appeared important for HIV-1 restriction activity as well, since substitutions of H216 resulted in lower restriction *in vivo*. Since kinetic analyses indicated smaller K_d at lower pH, we monitored substrate binding by taking quick NMR spectra right after mixing substrate with protein at pH 6.0. The disappearance of specific NMR signals delineated residues on the substrate-binding surface. The identified surface is consistent with prior NMR DNA-titration studies.[2,3]

References

1. Harjes S, Solomon W, Li M, Chen K-M, Harjes E, Harris RS and Matsuo H "Impact of H216 on the DNA binding and catalytic activities of the HIV restriction factor APOBEC3G". *Journal of Vilorogy*, *JVI.03173-12; published ahead of print 17 April 2013 doi:10.1128/JVI.03173-12*.
2. Chen K-M, Harjes E, Gross P-J, Fahmi A, Lu Y, Shindo K, Harris RS and Matsuo H (2008) "Structure of the DNA deaminase domain of the HIV-1 restriction factor APOBEC3G" *Nature*, 452, 116-119.
3. Furukawa A, Nagata T, Matsugami A, Habu Y, Sugiyama R, Hayashi F, Kobayashi N, Yokoyama S, Takaku H and Katahira M, (2009) "Structure, interaction and real-time monitoring of the enzymatic reaction of wild-type APOBEC3G" *The EMBO Journal*, 28, 440-451.

30. NMR Structure of Human Restriction Factor APOBEC3A: Substrate Binding and Enzyme Specificity

In-Ja L Byeon[1,2], Jinwoo Ahn[1,2], Mithun Mitra[3], Chang-Hyeock Byeon[1,2], Kamil Hercík[3] , Jozef Hritz[1], Lisa M Charlton[1,2], Judith G Levin[3] & Angela M Gronenborn[1,2]

[1]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA 15261, USA; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA; [3]Section on Viral Gene Regulation, Program on Genomics of Differentiation, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892-2780, USA

Human APOBEC3A (A3A) is a member of the A3 family of single-stranded DNA (ssDNA) deoxycytidine deaminases that play a role in the innate immune response to viral pathogens. A3A has a broad range of activities: it potently restricts LINE-1 retrotransposition, inhibits replication of retroviruses (e.g., HIV-1 and HTLV-1) and certain DNA viruses, degrades foreign DNA, and can function as a genomic DNA mutator. Here, we report the NMR solution structure of A3A, define the critical interface for interaction with its ssDNA substrates and present structural models for the A3A-ssDNA complexes. The overall structure of A3A is very similar to that of the A3G catalytic C-terminal domain, although structural details are different. Oligonucleotides containing the A3A-specific recognition site TTCA bind A3A 10-fold more tightly (K_d ~60 μM) than dCTP alone and the interaction surface includes residues that extend beyond the catalytic center. A3A also binds with similar affinity (~90 μM) to oligonucleotides containing the A3G-specific CCCA sequence. Real-time monitoring of the deamination reaction by NMR on A3A- and A3G-specific ssDNA substrates was conducted to determine the catalytic constants for A3A. Based on the structural and biochemical results, we propose a molecular mechanism that provides new insights into A3A functional activities.

31. Small Molecule Inhibitors of APOBEC3G DNA Cytosine Deaminase

Daniel A. Harki[†*], Margaret E. Olson[†], Angela L. Perkins[†], Daniel Abate Pella[†], Ming Li[‡], Anurag Rathore[‡], Michael A. Carpenter[‡], and Reuben S. Harris[‡]

[†]Department of Medicinal Chemistry, University of Minnesota, 717 Delaware Street S.E., Minneapolis, MN 55414, United States; [‡]Department of Biochemistry, Molecular Biology & Biophysics, University of Minnesota, 321 Church Street S.E., Minneapolis, MN 55455, United States

Nine single-stranded DNA cytosine-to-uracil (C-to-U) deaminases are expressed in human cells. Several of these members, including APOBEC3G (A3G), deaminate cDNA replication intermediates of retroviruses such as HIV-1, and these lesions lead to genomic strand G-to-A mutations.[1] This process may contribute to viral genetic diversity and allow HIV-1 to escape clearance from the immune system and resist antiviral drugs. We have hypothesized that small molecule inhibition of A3G may result in decreased viral fitness, which may confer antiviral effects (i.e., therapy by hypomutation).[2] We have utilized high-throughput screening and chemical synthesis to discover two unique chemical scaffolds that inhibit A3G-mediated cytosine deamination by covalently binding Cys321, which is located proximal to the enzyme active site.[3,4] More recent efforts have revealed an entirely new chemotype of A3G inhibitor that likely functions through a non-covalent mechanism of inhibition, yet similarly engages a region of A3G near Cys321. Our progress towards developing potent and selective A3G inhibitors for applications as mechanistic chemical probes and potential lead compounds for drug development will be presented.

References:
1. Albin, J. S.; Harris, R. S. *Expert Rev. Mol. Med.* **2010**, *12*, e4.
2. Harris, R. S. *Nat. Biotechnol.* **2008**, *26*, 1089.
3. Li, M.; et al. *ACS Chem. Biol.* **2012**, *7*, 506-517.
4. Olson, M. E.; et al. *ChemMedChem* **2013**, *8*, 112-117.

32. Cryo-Electron Tomography of Cryo-FIB Sectioned Mammalian Cells

Ke Wang[1,3], Korrinn Strunk[2,3], Gongpu Zhao[1], Yun Han[1], Jennifer L. Gray[2] and Peijun Zhang[1,2]

[1]Department of Structural Biology, School of Medicine; [2]Department of Mechanical Engineering and Materials Science, School of Engineering, University of Pittsburgh, Pittsburgh, PA 15260, USA

Cryo-electron tomography (cryo-ET) has enabled high resolution three-dimensional (3D) structural analysis of virus and cells and interactions between them; these studies, however, have largely been limited to very thin, peripheral regions of eukaryotic cells or to small prokaryotic cells. Recent efforts to make thin, vitreous sections using cryo-ultramicrotomy have been successful, however, this method is technically challenging and with many artifacts. Here, we show a simple and robust method for creating in situ, frozen-hydrated cell lamellas using a focused ion beam at cryogenic temperature (cryo-FIB), allowing access to any interior cellular regions of interest for high resolution structural analysis by cryo-ET. We demonstrate the utility of cryo-FIB with 3D cellular structures from both bacterial cells and large mammalian cells. The method will not only facilitate high-throughput 3D structural analysis of biological specimens, but is also broadly applicable to sample preparation of thin films and surface materials without the need for FIB “lift-out”.

33. A Small Molecule Inhibitor Targeting Capsid and Nuclear Import Blocks HIV-1 Replication

Atsuko Hachiya[1,2], Christie Pautler[1], Jennifer Moran[1], Sanath Janaka[1], Karen A. Kirby[1], Eleftherios Michailidis[1], Yee T. Ong[1], Shinichi Oka[3], Michael A. Parniak[4], Wataru Sugiura[2], KyeongEun Lee[5], Vineet N. KewalRamani[5], Kamalendra Singh[1], and Stefan G. Sarafianos[1,6]

[1]University of Missouri, Department of Molecular Microbiology & Immunology, Columbia, MO, 65211; [2]Clinical Research Center, National Hospital Organization Nagoya Medical Center, Department of Infectious Diseases and Immunology, Nagoya, 4600001, Japan; [3]AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, 1628655, Japan; [4]University of Pittsburgh, Department of Molecular Genetics & Biochemistry, Pittsburgh, PA, 15219; [5]National Cancer Institute, HIV Drug Resistance Program, Frederick, MD, 21702; [6]University of Missouri, Department of Biochemistry, Columbia, MO, 65211

HIV capsid (CA) has thus far been an unexploited target, therefore providing excellent opportunities for the discovery of new antiretrovirals that act by novel mechanisms of action. We screened a library of chemical compounds and identified 18E8, a small molecule that interferes with multimerization of HIV-1 CA. In cell-based assays, 18E8 showed broad antiretroviral activity. 18E8 exerts its antiretroviral activity by binding to HIV-1 CA, which was revealed by experiments for the selection of drug resistance in which an A105T mutation in CA confers resistance to the compound. In order to understand the mechanism of action of 18E8 and to determine the specific step of the viral replication cycle that it affects, we performed time-of-drug addition experiments that determined how long the addition of 18E8 could be postponed before observing a loss of antiviral activity. By comparing its relative position in the time scale to that of drugs that target reverse transcriptase (RT) and integrase, we demonstrated that 18E8 targets an early step in the HIV replication cycle, after reverse transcription. Consistent with these results, cell-cell fusion and RT-catalyzed primer extension assays showed that 18E8 did not affect the viral entry or RT steps. To further evaluate the level of different HIV DNA forms during the RT to integration steps, we performed qPCR to detect late RT products, 2-LTR circles, and integrated viral DNA. Surprisingly, although 18E8 targeted CA, it decreased the amount of integrated viral DNA. 18E8-resistant virus carrying A105T in CA improved the efficiency of integration. It has been reported that CA is required for events occurring after nuclear import that influence HIV-1 integration. Taken together, our data suggest that 18E8 acts during nuclear import and affects integration by interacting with CA, which is an unexpected function.

34. A Detailed Analysis of the Role of TNPO3 in HIV-1 Infection

Ilker Oztop[1], KyeongEun Lee[2], Goedele N Maertens[3], Peter Cherepanov[4], Vineet N KewalRamani[2], Alan Engelman[1]

[1]Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, MA, 02215; [2]HIV Drug Resistance Program, National Cancer Institute, Frederick, MD, 21702; [3]Department of Medicine, Imperial College London, UK; [4]Clare Hall Laboratories, London Research Institute, Cancer Research UK.

HIV-1 infection is dependent on transportin-3 (TNPO3 or TRN-SR2), a karyopherin beta family member that is responsible for nuclear transport of serine/arginine-rich (SR) proteins involved in mRNA splicing. Characterizing the infection block in cells depleted for TNPO3 has yielded disparate findings. Some groups have reported significant reductions in the formation of viral DNA 2-LTR circles, which is indicative of a block in the nuclear import of the preintegration complex (PIC), whereas others have reported normal levels of 2-LTR circle formation. 2-LTR circle assays can also detect products of HIV-1 autointegration, which can complicate the interpretation of PIC nuclear import phenotypes. Here we analyze total HIV-1 DNA levels in various cell fractions to assess the role of TNPO3 in PIC nuclear import independent of 2-LTR circle PCR assays.

Cleavage and polyadenylation specificity factor (CPSF) 6 is an SR-like protein that contains a C-terminal RS/RD domain. We previously showed that expression of a C-terminal truncation mutant of CPSF6, CPSF6-358, which is missing the RS/RD domain, restricts HIV-1 infection at the step of nuclear import without affecting the intrinsic activity of the PIC to integrate into a target plasmid DNA in vitro. We also showed that a single point mutation in capsid (CA), N74D, overcame CPSF6-358 restriction and TNPO3 dependency during HIV-1 infection, and diminished the binding of CPSF6-358 protein to recombinant CA-nucleocapsid (CA-NC) tubes that mimic the surface structure of intact viral cores. Although TNPO3 also binds CA-NC tubes, the N74D change does not alter this interaction. We have also expanded the size of our panel of HIV-1 CA mutant viruses, and correlate their sensitivity to CPSF6-358 restriction and dependence on TNPO3 with the ability of the respective CA-NC tubes to bind the two factors.

The correlation between TNPO3 dependency and sensitivity to CPSF6-358 mediated restriction of several HIV-1 CA mutant viruses, and the loss of the RS domain from CPSF6-358, prompted us to test for a direct interaction between CPSF6 and TNPO3. Here we show specific binding between recombinant TNPO3 and the RS domain of CPSF6. The interaction is down modulated by RanQ69L-GTP, indicating that CPSF6 is a bona fide import substrate of TNPO3. Mutagenesis experiments to fine-tune the points of interaction are underway. Models that discuss the role of TNPO3 in HIV-1 infection will be discussed.

35. Modeling the HIV-1 Intasome

Barry C. Johnson[1], Mathieu Métifiot[2], Yves Pommier[2], and Stephen H. Hughes[1]

[1]HIV Drug Resistance Program, Frederick National Laboratory, Frederick, MD 21702, USA and [2]Laboratory of Molecular Pharmacology, National Cancer Institute, Bethesda, MD 20892, USA

Retroviral integrase (IN) is the enzyme responsible for inserting the viral DNA (vDNA) into the DNA of a host cell (hDNA). Recently solved crystal structures of the prototype foamy virus (PFV) intasome, including an IN tetramer with vDNA and hDNA mimics, have allowed for improved modeling of the HIV-1 intasome. Last year, we presented an initial model of the HIV-1 intasome that showed a helix-helix dimer contact between two catalytic core domains. Including this structural element in the model results in a rearrangement of the C-terminal domain compared to previous published peptide structures. Further testing has supported these observations. Mutant single-round vectors were generated based on the predictions from the model and assayed for viral infectivity, vDNA synthesis, 2-LTR circle formation, and core morphology. These results support the proposed structure. The model is consistent with previously published biochemical data and the new data presented here, suggesting it may serve as a tool to develop novel allosteric integrase inhibitors.

36. Apo-Architecture and Assembly of a DNA Invasion Machine: HIV Integrase and Its Inhibition

Ravi S.Bojja, Mark D. Andrake, George Merkel, Steven Weigand, Roland L. Dunbrack, Jr., and Anna Marie Skalka

Fox Chase Cancer Center, Philadelphia, PA

We have applied small angle x-ray scattering and protein cross-linking coupled with mass spectrometry to determine the architectures of full-length HIV integrase (IN) dimers in solution. By blocking interactions that stabilize either a core-core domain interface or N-terminal domain intermolecular contacts, we show that full-length HIV IN can form two dimer types. One is an expected dimer, characterized by interactions between two catalytic core domains. The other dimer is stabilized by interactions of the N-terminal domain of one monomer with the C-terminal domain and catalytic core domain of the second monomer as well as direct interactions between the two C-terminal domains. This organization is similar to the “reaching dimer” previously described for wild type ASV apo IN and resembles the inner, substrate binding dimer in the crystal structure of the PFV intasome. Results from our small angle x-ray scattering and modeling studies indicate that in the absence of its DNA substrate, the HIV IN tetramer assembles as two stacked reaching dimers that are stabilized by core-core interactions. These models of full-length HIV IN provide new insight into multimer assembly and suggest additional allosteric approaches to stabilize alternate conformations for enzyme inhibition.

Bojja RS, *et al*, Architecture and Assembly of HIV Integrase Multimers in the Absence of DNA Substrates. J Biol Chem. 2013 Mar 8;288(10):7373-86

37. The A128T Resistance Mutation Reveals Aberrant Protein Multimerization as the Primary Mechanism of Action of Allosteric HIV-1 Integrase Inhibitors

Lei Feng[1], Nivedita Jena[2], Amit Sharma[1], Alison Slaughter[1], Jacques J. Kessl[1], Alan Engelman[3], James R Fuchs[2], Mamuka Kvaratskhelia[1]

[1]The Ohio State University, Center for Retrovirus Research, College of Pharmacy , Columbus, OH, 43210, [2]The Ohio State University, Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, Columbus, OH, 43210, [3]Dana-Farber Cancer Institute, Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, & Department of Medicine, Harvard Medical School, Boston, MA, 02115

HIV-1 integrase (IN) is an important therapeutic target as its function is essential for viral replication. Allosteric HIV-1 IN inhibitors (ALLINIs) are a new class of anti-HIV-1 agents that exhibit a multimodal mechanism of action by 1) allosterically modulating IN multimerization, and 2) interfering with IN-LEDGF/p75 binding. Selection of viral strains under ALLINI pressure has revealed an A128T substitution in HIV-1 IN as a primary mechanism of resistance. A128 is located at the IN dimer interface in the pocket occupied by ALLINIs or LEDGF/p75.

Here, we have elucidated the structural and mechanistic basis for this resistance. The A128T substitution did not affect the hydrogen bonding between ALLINI and IN that mimics the IN-LEDGF/p75 interaction, but instead altered the positioning of the inhibitor at the IN dimer interface. Consequently, the A128T substitution had only a minor effect on the IN-LEDGF/p75 binding, but markedly altered the multimerization of IN. ALLINIs promoted aberrant, higher order multimerization of wild type but not A128T IN. Accordingly, wild type IN catalytic activities and HIV-1 replication were potently inhibited by ALLINIs, whereas the A128T substitution in IN resulted in significant resistance to the inhibitors in both *in vitro* and in cell culture assays. The differential multimerization of WT and A128T INs induced by ALLINIs correlated with the differences in infectivity of HIV-1 progeny virions. Taken together, we conclude that ALLINIs primarily target allosteric integrase multimerization rather than IN-LEDGF/p75 binding. Our findings provide the structural foundations for developing improved ALLINIs with increased potency and decreased potential to select for drug resistance.

38. Hydrogen Bond Interactions of Inhibitory Molecules with Amino Acids of HIV-1 Integrase within a 4.0Å Radius of the Active Site: Inhibitors Classified as Drug-like and Nondrug-like According to Lipinski's "Rule of Five"

Julie B. Ealy, Sarah Israel, Talia Katz, Robert Huff, and Paolo Flauta

Penn State University Lehigh Valley, Center Valley, PA

Computational software ICM Pro (MolSoft, LaJolla, CA) was used to assess the hydrogen bond interactions of inhibitors of human immunodeficiency virus type 1 (HIV-1) integrase in the active site of the enzyme (Protein Data Bank entry 1BIS). Many of these inhibitors have drug-like properties that follow Lipinski's rule of five, that were introduced for predicting oral bioavailability. These rules include: molecular mass less than 500 g/mole, log P (the partition coefficient, defined as the ratio of the concentration of a drug in octanol to its concentration in water) less than 5, and no more than 5 hydrogen bond donors and 10 hydrogen bond acceptors (reviewed in J.B. Ealy and V. Kvarta, J. Chem. Ed. 83:1779, 2006). However, some integrase inhibitors do not follow these rules and are considered nondrug-like.

39. Allosteric Integrase Inhibitor Potency is Determined Through the Inhibition of HIV-1 Particle Maturation

Kellie A Jurado[1], Hao Wang[1], Lei Fang[2], Jacques J Kessl[2], Yasuhiro Koh[1], Weifeng Wang[1], Allison Ballandras-Colas[1], Pratiq A Patel[3], James R Fuchs[3], Mamuka Kvaratskhelia[2], Alan Engelman[1]

[1]Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, MA 02215; [2]Center for Retrovirus Research and Comprehensive Cancer Center, College of Pharmacy and [3]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210

Integration of HIV-1 is essential for virus replication and is mediated by a homotetrameric complex of the viral protein integrase (IN) in association with the ends of linear viral DNA. HIV-1 integrates within actively transcribed genes, a trait that is mediated by the cellular host cofactor LEDGF/p75. LEDGF/p75 acts as a bimodal tether that constitutively binds chromatin through N-terminal elements and engages IN through a C-terminal IN-binding domain at a cleft formed through the dimerization of the IN catalytic core domain, a region that has recently been validated as a target for allosteric IN inhibitors (ALLINIs).

Previous *in vitro* work suggested that ALLINIs work through the disruption of two integration-associated functions, IN catalysis and the IN-LEDGF/p75 interaction. We now demonstrate that ALLINI potency is accounted for during the late phase of HIV-1 replication where the inhibitors block the formation of the viral core, converting the normally electron-dense conical core to an eccentric phenotype with electron-dense material situated between the translucent core and viral membrane. ALLINI treatment did not alter levels of viral RNA or protein incorporation, or affect entry into drug-free target cells. Reverse transcription and integration were by contrast both defective. ALLINI treatment therefore recapitulates the phenotype of class II IN mutations.

ALLINIs retained full potency when IN function was supplied in trans by Vpr-IN, suggesting that IN is the drug target during virus production. ALLINI potency was independent of the level of LEDGF/p75 expression during viral egress, but increased significantly when LEDGF/p75 was knocked down during the acute phase of HIV-1 replication. LEDGF/p75, therefore, competes with ALLINIs for IN binding during viral ingress, but apparently not during egress. ALLINI treatment enhanced higher-order oligomerization of both purified IN protein and virion-associated IN, suggesting disregulated IN multimerization as the putative mechanism of ALLINI inhibition. The hypersensitivity of IN to small molecules during the late phase of HIV-1 replication unveils a pharmacological Achilles' heel for exploitation in clinical ALLINI development.

40. Novel Approach to Developing HDAC Benchmarking Sets – An Aid to SBDD of Isoform-Selective HDAC Inhibitors for Disrupting HIV Latency

Terry-Elinor Reid[1,2],Jie Xia[1,2,3],Napoleon Manian[2,4], Liangren Zhang[3], Xiang Simon Wang[1,2]

[1]*Molecular Modeling and Drug Discovery Core for District of Columbia Developmental Center for AIDS Research (DC D-CFAR);* [4]*Center of Excellence College of Pharmacy; Laboratory of Cheminfomatics and Drug Design,* [2]*Department of Pharmaceutical Sciences, College of Pharmacy, Howard University, Washington, District of Columbia 20059*; [3]*State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100191*

Histone deacetylases (HDACs) are a family of enzymes that regulate epigenetic gene expression via post-transcriptional modification of histones. The mechanism by which this occurs is very well known and involves the removal of acetyl groups form the ε-amino group of lysine residues on histones resulting in condensation of the chromatin structure by DNA, consequently silencing that segment of DNA thereby suppressing gene expression. HDACs are promising targets for the treatment of cancer, neurodegenerative disorders and more recently Human Immunodeficiency Virus (HIV). The endeavor to find antiretroviral therapy for the treatment of over 50 million people worldwide infected with Human Immunodeficiency Virus (HIV) is an ongoing task and complete eradication of HIV remains a scientific challenge due to the latent infection of dormant HIV. HIV latency is maintained through synergistic mechanism of class I HDACs which include HDAC isoforms 1, 2, and 3. It has been reported that inhibition of class I HDACs will activate HIV long terminal repeat and consequently cause the escape of HIV latency. One major issue of current HDAC inhibitors, such as TSA and SAHA, is that they target multiple HDAC isoforms which results in unspecific inhibition thus lead to adverse drug reaction and toxicity. Hence there is a critical need to develop highly isoform-selective HDAC inhibitors. During our recent efforts to rationally design isoform-selective HDAC2 inhibitors using novel structure–based Drug Design (SBDD) approaches developed by our group, we addressed the need for challenging HDAC benchmarking datasets with success. SBDD entails docking and scoring small molecules libraries to find compounds with high binding affinity to an active site of protein target. To achieve this goal docking performance is normally evaluated and validated by benchmarking sets. Hence, the quality of the benchmarking set is of paramount importance and the unbiased sets can ensure more objective and accurate evaluation. Herein we report a novel and robust approach to building least-biased benchmarking set for HDACs. This approach involves the use of series of filtering strategies based on physiochemical properties matching between decoys and ligands, topology-based similarity exclusion, followed by Leave-One-Out (LOO) validation. The whole protocol ensures good quality of benchmarking sets with less artificial enrichment and analogue bias than those of publically available benchmarking data sets. Upon validation of our novel approach we employed it to build benchmarking sets for various HDAC isoforms. The results of property distribution curves indicate that the properties of our decoy compounds match well with true ligands; the LOO validation ROC curves are close to the random distribution with an AUC value close to 0.50 which is an indication of low bias within the decoy set. An unique feature of our benchmarking sets is their broad application to the evaluation of ligand-based drug design (LBDD) approaches as well.

41. Development of Isoform-Specific Inhibitors of HDACS to Probe Expression of Latent HIV-1 Genomes

Garland R Marshall[1], Sergio Valente[3], Rino Ragno[3], Antonello Mai[3], Lee Ratner[2]

[1]Washington University, Biochemistry & Molecular Biophysics, St. Louis, MO, 63110, [2]Washington University, Medicine/Molecular Oncology, St. Louis, MO, 63110, [3]Sapienza Universita di Roma, Dipartimento di Chimica e Tecnologie del Farmaco, Rome, 00185, Italy

Histone deacetylase inhibitors (HDACIs) have been shown to reactivate the expression of the HIV genome in infected cells. The HDACIs used in these studies inhibited multiple isoforms of the eleven zinc-based HDACs expressed by the human genome. Huber et al. (JBC, 2011) correlated the efficacy of a variety of non-specific HDACIs with their ability to overcome HIV latency in J89GFP cells, a Jurkat T-cell line that contains a stably integrated, full-length HIV-1 provirus (strain 89.6) with an enhanced green fluorescent protein (EFGP) reporter incorporated into the viral genome. The ability to overcome HIV latency correlated best with the potency of HDACIs toward HDAC3. We have investigated the ability of more isoform-selective HDACIs to overcome latency in a different cell line, J-Lat 10.6 cells. In particular, two phenylisoxazole inhibitors, first reported by Kozikowski et al. as highly selective as inhibitors of HDAC3 and HDAC6 were as effective as apicidin and oxamflatin (two non-selective HDACIs) at comparable doses. Two novel isoform-specific inhibitors of HDAC6 have recently been discovered and are bing examined for their effects on HIV latency.

We have developed a heuristic 3D-QSAR model, COMBINEr Silvestri et al., JCIM, 2012), for predicting the relative activities of novel compounds for each of the eleven zinc-based HDACs based on docking of the compound to the crystal structures or homology models of the isozymes. This predictive model is being iteratively refined as more structural information on HDACI/isozyme complexes and structure-activity data becomes available. It is clear that dynamic acetylation plays a significant role in control of HIV latency, and that inhibition of HDACs can cause expression of the HIV genome (Shriakawa et al., Trends Microbiology, 2013).

The motivation to determine the most essential therapeutic strategy is based on the recognition of potential off-target side effects in inhibition of enzymes that are part of the epigenetic control of gene expression. As dynamic acetylation of over 1700 non-histone proteins in cells has been demonstrated by proteomics, it is quite feasible that release of latency and expression of the HIV genome depends on the acetylation of Tat and other factors, and their subsequent interaction with the elongations factors on the AFF4 scaffold (Chou, PNAS, 2013).

42. The AFF4 Scaffold Binds Human P-TEFb Adjacent to HIV Tat

Ursula Schulze-Gahmen[1], Heather Upton[1], Andrew Birnberg[1], Nevan J. Krogan[2,3,4], Qiang Zhou[1], Tom Alber[1,5]

[1]Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720 USA; [2]Department of Cellular and Molecular Pharmacology, University of California, 1700 4th Street, San Francisco, CA 94158 USA; [3]California Institute for Quantitative Biosciences, QB3, San Francisco CA 94158 USA; [4]J. David Gladstone Institutes, San Francisco, CA, 94158, USA; [5]California Institute for Quantitative Biosciences, QB3, Berkeley, CA 94720 USA

Human positive transcription elongation factor b (P-TEFb) phosphorylates RNA polymerase II and regulatory proteins to trigger elongation of specific gene transcripts. At key genes—including the integrated HIV genome—P-TEFb functions as part of a super elongation complex (SEC), a large assembly organized on a flexible scaffold in the AF4 family. The HIV-1 Tat protein selectively recruits SECs containing AF4 proteins AFF1 or AFF4. To explore the basis for this specificity and determine if scaffold binding alters P-TEFb conformation, we determined the cocrystal structure of a tripartite complex containing the functional recognition regions of P-TEFb and AFF4. AFF4 meanders over the surface of the cyclin T1 (CycT1) subunit of P-TEFb, making no stable contacts with the CDK9 kinase subunit. Amino acid substitutions in the interface reduce CycT1 binding and AFF4-dependent transcription. Unexpectedly, AFF4 is positioned to make direct contacts with HIV1 Tat, and Tat enhances P-TEFb affinity for AFF4. These studies define the mechanism of scaffold recognition by P-TEFb and reveal an unanticipated intersubunit pocket on the AFF4 SEC that potentially represents a target for therapeutic intervention against HIV/AIDS.

43. Towards Developing a Structure Based Mechanism of Splicing Repression by hnRNP A1 at ssA7 on HIV-1

Jeffrey D. Levengood, Carrie Rollins, and Blanton Tolbert

Case Western Reserve University, Department of Chemistry, Cleveland, OH 44106

Alternative splicing of the HIV-1 genome is necessary for translation of the complete viral proteome. Host proteins, such as hnRNP A1, are used to regulate splicing at the various donor and acceptor sites along the viral genome. One such site regulated by hnRNP A1 is the conserved 3' acceptor splice site A7 (ssA7). Silencing of splicing at this site is necessary in order to retain the Rev Responsive Element (RRE) in the adjacent tat/rev intron. The RRE is responsible for nuclear export of unspliced and partially spliced transcripts. .

Our research seeks to clarify the binding determinant of hnRNP A1 on ssA7 by developing a structural model that will correlate ssA7 structure to its splicing function. For this model we are using isolated domains of both hnRNP A1 and ssA7. The protein UP1 is composed of the two RRM domains of hnRNP A1. For ssA7, SL3 of the three stem loop structure is examined as this contains a high affinity UAG binding site for hnRNP A1. We previously solved the 3D solution structure of SL3 by NMR and found the UAG is located in a terminal heptaloop.

The points of interaction for UP1 and SL3 were probed with both NMR and Isothermal Titration Calorimetry (ITC). Chemical shift mapping by NMR was done to determine which residues of UP1 may bind SL3. For determining where UP1 binds SL3, residues of the terminal heptaloop were mutated. The binding of UP1 to these SL3 mutants was then examined with ITC. The results of these two sets of experiments were then used in HADDOCK modeling to determine a possible UP1:SL3 co-structure.

44. Structural Fidelity and NMR Relaxation Analysis in a Prototype RNA Hairpin

George M. Giambaşu,[2] Niel Henriksen,[1] Thomas E. Cheatham, III,[1] Darrin M. York[2] and David A. Case[2]

[1]Department of Medicinal Chemistry, College of Pharmacy, University of Utah, 2000 East 30 South Skaggs 105, Salt Lake City, UT 84112; [2]BioMaPS Institute for Quantitative Biology and Department of Chemistry and Chemical Biology Rutgers University 174 Frelinghuysen Rd, Piscataway, NJ 08854

Hairpins represent one of the most encountered RNA motifs and are known for their stability and for their role in RNA folding and function. We present a set of 32 explicit solvent simulations (each of 200 ns duration) of an UUCG RNA hairpin, analyzing the stem and loop structures and NMR observables from simulations. The simulations reproduce the temperature and ionic strength conditions used to derive NMR parameters and use AMBER molecular mechanics force fields setups best suited (currently) for RNA conformations, water diffusion properties and water mediated ion-ion interactions. The current setup yields overall tumbling times that are lower than the experimental values (determined from NMR relaxation) by 20-30% but follow the same trend with temperature and ionic strength. About three-quarters of the simulations maintain the canonical UUCG turn motif seen in NMR structures of this sequence and many others; the remaining simulations irreversibly shift G8 to an exposed position. Learning how to prevent this change may help in other situations where current simulation protocols fail to give good results for RNA single stranded regions. Simulations using the current *ff10* force field are notably superior to those using earlier force fields. The results provide new insight into how NMR relaxation and residual dipolar coupling measurements can aid in structural analysis.

45. Advances in NMRViewJ for Analysis of NMR Spectra of RNA

Shawn Barton[1,2] Xiao Heng[1,2], Michael F. Summers[1,2] & Bruce A. Johnson[2,3]

[1]Howard Hughes Medical Institute, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250; [2]Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250; [3]One Moon Scientific, Inc., 839 Grant Ave., Westfield, NJ 07090

NMRViewJ is one of the most widely used programs for the visualization and analysis of macromolecular NMR spectra. Development of specialized features in NMRViewJ has focussed primarily on its application to spectra of proteins. We describe here recent work intended to develop NMRViewJ into a powerful tool for analysis of the NMR spectra of RNA. In order to facilitate the assignment and validation of RNA chemical shifts we developed a predictive model that uses as inputs the primary sequence and secondary structure attributes (helices, loops, bulges etc.). The model was trained using chemical shifts deposited at the BMRB and performs very well. The model was then integrated into NMRViewJ so that the user can enter an RNA sequence and secondary structure and immediately generate predicted values for the chemical shifts. Based on these predicted shifts, and experiment-specific connectivities, we can then generate a simulated peak list with a network of connections. An interactive tool allows the user, while observing the matching of a whole network of peaks, to adjust the simulated peak list to coincide with measure peak positions. We also describe our ongoing work to incorporate structural information in the RNA analysis. The chemical shift prediction tool is being extended to incorporate 3D structural information, including ring-current shifts, and structure calculation methods are being incorporated to provide structural feedback early in a project.

46. SHAPE Analysis of HIV Rev Response Element (RRE) *In Vitro* and *In Vivo*

Yun Bai[1], Jennifer A. Doudna[1,2,3,4]

[1]Department of Molecular and Cell Biology, [2]Howard Hughes Medical Institute, [3]Department of Chemistry, University of California, Berkeley, California 94720, USA; [4]Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA

The HIV Rev response element (RRE) is a highly structured RNA segment within the HIV genome. It is well established that this region interacts with multiple copies of the HIV Rev protein to facilitate nuclear export of partially spliced and unspliced viral RNA. However, once in the cytoplasm, the RRE needs to dissociate from Rev to allow efficient translation or packaging of the viral RNA. And it is unclear when and how this process occurs.

Here, we are using selective 2' hydroxyl acylation analyzed by primer extension (SHAPE) based chemical probing assays to access the structural changes of the RRE RNA when it encounters either the HIV Rev multimer or host factors. Using *in vitro* time-resolved SHAPE assays, we obtained a series of SHAPE snapshots during the course of RRE-Rev RNP formation, which provided insights into the kinetic intermediates of this multimerization process. Using *in vivo* SHAPE analysis, we observed that although most of the RRE showed a SHAPE profile similar to that obtained *in vitro*, critical Rev binding regions are protected in the absence of Rev. This reveals an RRE conformation that may no longer support Rev binding and indicates that Rev multimer on the RRE could be replaced by host factors after nuclear export.

47. Characterization of the Interactions Between HIV Rev Protein and Host RNA Helicase DDX21 *In Vitro*.

Li Zhou and James R Williamson

Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA, 92037

The Rev protein of HIV-1 binds to the Rev Response Element (RRE) located in the *env* region of incompletely spliced HIV mRNA and promotes nuclear export of intron containing viral transcripts. This step is one of the benchmarks for the transition from early to late viral life cycle and viral infectivity. HIV utilizes many host protein factors during these processes. In an effort to identify these host protein factors, a previous proteomic and cell biology study characterized several DEAD/H box RNA helicases linked to the Rev/RRE nuclear export pathway *in vivo*. In particular, DDX21 silencing experiments suggested that DDX21 is directly involved in the Rev-RRE dependent nuclear export in the late stage of HIV infection, and it might play a role in the assembly of Rev-RRE complexes. To begin understanding what role DDX21 plays in Rev/RRE functions we explored their direct interaction. Using recombinant DDX21 and Rev proteins purified from *E. coli,* we mapped the interaction sites with Rev via various *in vitro* biochemical binding assays and proposed a model of DDX21/Rev interaction. Further, we established the minimal domains of DDX21 required for the RNA-dependent ATPase activity, and the Michaelis-Menten constants of DDX21. Our data provide insights into the mechanistic role of DDX21 in HIV Rev/RRE dependent nuclear export pathway, and has implications in DEAD box protein-dependent nuclear export of RNA in general.

48. Toward Understanding the Role of Human DEAD-Box Protein 1 (DDX1) in Rev-Dependent Export of HIV-1 RNA

J. A. Hammond, Li Zhou, David P. Millar, Jamie R. Williamson

The Scripps Research Institute Department of Molecular Biology, 10550 North Torrey Pines Road, La Jolla, California, 92037

Nuclear export of partially and fully unspliced HIV-1 transcripts is dependent on viral protein Rev binding to, and subsequently oligomerizing on, the Rev Response Element (RRE) located within the viral RNA. Recently, a number of DEAD-box helicases (DDXs) were shown to be associated with Rev *in vivo*, affecting various steps in the HIV viral maturation. DDX1 was the first member of this family to be recognized as a Rev interacting partner and subsequent data has pointed to this factor acting during nucleo-cytoplasmic shuttling. Here we demonstrate that the interaction between DDX1 and Rev is specific and of high affinity, being localized to the N-terminus of the conserved DEAD-box domain. Further, in other known Rev-interacting DDX proteins this domain shows a similar affinity for Rev, suggesting a conserved mechanism of binding. Preliminary single-molecule data suggests DDX1 acts not just by interacting with Rev but also with the RRE RNA. Here we present biochemical and biophysical data characterizing the binding between these partners and the effects this binding has on the HIV-1 RNA secondary structure. A model is presented to describe a possible role and mechanism for DDX1 in nucleo-cytoplasmic shuttling.

49. Imaging DDX1 Interaction with HIV-1

Ammeret Rossouw and David Grunwald

UMass Medical School, RNA Therapeutics Institute, Worcester, Massachusetts

There are significant data that DDX1, a DEAD-box helicase with ATPase activity, participates in the Rev assisted export of HIV-1 RNA genomes. It has been shown that DDX1 is both a critical cellular cofactor for Rev function and that DDX1 is directly responsible for maintenance of the proper sub-cellular distribution of Rev. Additionally, it was also been shown that DDX1 promotes Rev oligomerization in vitro.

Of particular interest to us is the spatial and temporal landscape of the Rev-RRE and DDX1 interactions. While it seems that DDX1 associates with the transcript and promotes Rev oligomerization it is not clear if DDX1 then releases from the transcript or perhaps shuttles the transcript to the NPC and then releases or even perhaps shuttles with the transcript across the nuclear pore?

Using single molecule microscopy, superregistration, high-tech labeling methods for both HIV-1 proteins, host factor DEAD box helicases and HIV-1 RNA genomes, as well as an extensive library of DDX1 mutants compiled and analyzed by the Gerace lab we aim to expand our knowledge of the nuclear interactions of HIV-1 genomes with the DDX1 helicase by providing single molecule spatial and temporal information specifically aiming to cross-correlate the binding state of DDX1 to the HIV-1 genome within the nucleus, focusing on three aspects of HIV-1 genome life cycle, i.e. at the transcription site of the integrated pro-virus, during intra-nuclear “transport” of the HIV-1 genome (i.e. diffusion to the nuclear periphery); and export of the HIV-1 genome across the nuclear envelope. We will monitor, in vivo, DDX1 helicase's binding state on the HIV-1 RNA during and after transcription, in a Rev dependent manner, and whether these binding states are modulated at the NPC.

In our preliminary data we describe the microscope and cellular setup and show in preliminary results that while the system needs optimization, we have a working model with which to continue.

50. Single-Molecule Studies of HIV-1 Rev Assembly on the Rev Response Element

Rajan Lamichhane[1], John Hammond[1], Rebecca Filbrandt[2], Souad Naji[2], Larry Gerace[2], James Williamson[1] and David P. Millar[1]

[1]Department of Integrative Structural and Computational Biology, and [2]Department of Cell and Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037 USA

The HIV-1 Rev (Regulator of Expression of Virion) protein activates nuclear export of unspliced and partially spliced viral mRNAs, which encode the viral genome and the genes encoding viral structural proteins. Rev interacts with a highly conserved region, the Rev Response Element (RRE), located within the viral mRNA. Initially, a single Rev monomer binds to stem loop IIB of the RRE, whereupon additional Rev monomers are recruited to the RRE through a combination of RNA-protein and protein-protein interactions, resulting in the formation of a functional nuclear export complex. In addition, several cellular proteins, such as the DEAD box helicases DDX1 and DDX21 are known to be required for efficient Rev function *in vivo*, although their precise role is unknown. In this study, a novel two-color single-molecule fluorescence spectroscopic method was used to visualize oligomeric assembly of Rev on the RRE with single monomer resolution. Binding of multiple fluorescently labeled Rev monomers to a single immobilized and labeled RRE molecule was observed in real-time and the event frequencies and corresponding binding and dissociation rates for the different Rev-RRE stoichiometries were determined. We are also using the two-color assembly assay to dissect the mechanism by which DDX1 promotes oligomerization of Rev on the RRE. As a complement to the *in vitro* studies, a new method, single-molecule pull down, was used to isolate Rev-RRE-host complexes directly from mammalian cells and to determine their stoichiometry before and after export from the nucleus to the cytoplasm. Together, these studies are revealing new information about the pathway of oligomeric Rev-RRE assembly and the role played by cellular cofactors of HIV-1.

51. Evidence that the Rous Sarcoma Virus Gag Protein Captures its Genome in the Nucleus

Rebecca J. Kaddis[1], Estelle Chiari-Fort[1], Darrin V. Bann[1], Breanna L. Rice[1], Matthew S. Stake[1], and Leslie J. Parent[1, 2]

Penn State College of Medicine, [1]Department of Medicine, and [2]Department of Microbiology and Immunology, Hershey, PA 17033

The retroviral Gag polyprotein selects genomic RNA for encapsidation through a high-affinity interaction with the psi (Ψ) packaging sequence in the 5' leader sequence. However, the mechanism by which Gag finds the genomic RNA amidst the sea of cellular RNAs in the infected cell is not well understood. For Rous sarcoma virus (RSV), genetic and biochemical evidence from our laboratory suggests that Gag traffics through the nucleus to select genomes for packaging. To dissect the mechanism used by Gag to efficiently locate viral RNA in the nucleus, we directly visualized Gag-RNA interactions by replacing the RSV *src* gene with 24 copies of RNA stem-loops from the MS2 bacteriophage to create an infectious proviral construct called RSV-24x. When RSV-24x was co-expressed with the MS2-YFP coat protein, fluorescently-labeled viral RNAs localized to discrete foci in the nucleus, cytoplasm, and at the plasma membrane.

To visualize Gag-RNA interactions, we expressed fluorophore-tagged Gag with RSV-24x and MS2-YFP. Under steady-state conditions, wild-type Gag and RSV-24x RNA co-localized in discrete foci at the plasma membrane where budding occurs. Furthermore, time-lapse microscopy in living cells revealed that a small population of Gag co-localized with the viral RNA at punctate foci in the nucleus. Due to the transient nuclear trafficking of Gag, we utilized a nuclear export mutant of Gag (Gag.L219A) to further study the co-localization of Gag and viral RNA in the nucleus. Quantitative 2D analysis revealed that 55% of viral RNA foci co-localized with Gag.L219A foci. To determine whether co-localization between RSV-24x and Gag.L219A was Ψ-dependent, we deleted MΨ (deltaMΨ-24x) and were surprised to find that the mutant RNA colocalized with Gag to a similar degree as the wild-type RNA (49%), even though deltaMΨ-24x was not infectious. Furthermore, non-viral RNA containing 24 copies of the MS2 stem-loops partially co-localized with Gag.L219A (16%) in the nucleus. These data suggest that Gag may interact nonspecifically with cellular mRNAs in the nucleus.

To test the idea that Gag binds to RNA co-transcriptionally, newly transcribed RNAs were labeled with 5-fluorouridine, and we found that the nascent transcripts co-localized with Gag.L219A at a frequency comparable to the non-viral RNA (15%). Together these data suggest that Gag traffics to sites of active transcription to sample mRNAs as they are synthesized. Sending Gag to the site of RNA synthesis has two major advantages that would increase the efficiency of genome recognition: (i) the unspliced viral RNA is at a high local concentration; and (ii) the genome could be captured prior to its engagement with splicing factors, ensuring the encapsidation of unspliced RNA.

52. Investigate the Dimerization Mechanism of the 5' Leader RNA Genome in Simian Immunodeficiency Virus

Thao Tran, Jennifer Bohn, Michael Summers Ph. D.

Howard Hughes Medical Institute and Chemistry/Biochemistry Department, University of Maryland, Baltimore County, MD

The dimerization process, where two genomic RNA copies are non-covalently linked and selected for packaging, is essential for viral replication. However, the mechanism is not well understood. Therefore, this study investigates the dimerization mechanism of the 5' leader genomic RNA (5'-L) of Simian Immunodeficiency Virus from chimpanzees (SIVcpz), a comparable animal model to HIV-1.

The 5'-L primary sequences between SIVcpz and HIV-1 are 70% identical. RNAstructure and Mfold indicated that the 5'-L secondary structures of SIVcpz and HIV-1 have the following similar elements: *trans*-activation region (TAR), polyadenylation signal (poly A), primer binding site (PBS), dimer initiation site (DIS), and gag start codon (AUG) stem-loops. Based on phylogenic analysis, primary sequence and secondary structures comparisons, we hypothesize SIVcpz 5'-L can utilizes a RNA structural switch similar to HIV-1 5'-L in order to regulate its dimerization in genome packaging.

To test our hypothesis, we first focus on the unique 5 (U5), palindromic sequence at DIS loop, and AUG regions within both SIVcpz and HIV-1 5'-L. Our initial gel results indicate that dimerization is possibly induced by U5:AUG interaction. This finding supports our hypothesis for SIVcpz 5'-L dimerization mechanism.

53. Structural Characterization of the 30 kDa HIV-1 RNA Dimerization Initiation Site by Cryo-EM

Rossi Irobalieva[1,2], Xiao Heng[3], Steven J. Ludtke[1,2], Michael F. Schmid[1,2], Michael F. Summers[3], Wah Chiu[1,2]

[1]Graduate Program in Structural and Computational Biology and Molecular Biophysics, Baylor College of Medicine, Houston, TX; [2]National Center for Macromolecular Imaging, Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX; [3]Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, Baltimore MD

Electron cryo-microscopy (cryo-EM) is a powerful structural technique that can now routinely reach 3-5 Å resolution for large, homogeneous specimens, such as protein machines and viruses. The advantage of cryo-EM is that prior to imaging, specimens are frozen instantaneously in liquid ethane, which allows them to retain a near-native hydrated state. Here we use single particle cryo-EM to visualize the dimerization initiation site (DIS) of HIV-1 RNA. At merely 88 nucleotides (~30 kDa), this is the smallest sample to date studied by cryo-EM.

One of the biggest challenges in working with specimens under 100 kDa is the low signal to noise ratio of the raw data. Sufficient signal is crucial for particle identification and orientation determination. One way to obtain higher contrast is to increase the number of electrons hitting the specimen. However, the drawback of this approach is that a high electron dose can destroy the higher resolution features of the sample. In order to increase the contrast, while preserving high-resolution information, we employed a new direct electron detector camera system, which has provided us with the ability to acquire a series of two-dimensional images of the same area and within a relatively long exposure time period (2.5 seconds) and a higher dose (75 e/Å^2). The entire stack of images aligned and averaged, yields high contrast, improving particle identification. Furthermore, during the three-dimensional reconstruction, we can focus on a particular subset of the image stack and remove data where the higher resolution features are compromised due to radiation damage. Our current results of the DIS RNA show a three-dimensional structure that is consistent in size and shape with the results obtained by NMR. We are able to independently resolve features such as the bend of the molecule and detect the pitch of the RNA helix. The validity and resolution are assessed with the latest gold standard criteria as established in the cryo-EM field.

Recent technology advances, such as those used in our study, have made it possible to push the limits of cryo-EM, opening the door to opportunities to study specimens much smaller than those traditionally considered. Cryo-EM is a powerful tool and combined with other structural techniques can help unravel the mystery around the structural behavior of critical regions such as the DIS. In the future, we plan to use this technique to also study RNA-protein interactions, providing further insight into the mechanics behind the processes of HIV genome dimerization and packaging.

54. Host RNA Helicase Incorporated into HIV-1 Virions: Chaperone Activity Promotes the Infectivity of Progeny Virions

Ioana Boeras[1], Amit Sharma[2] and Kathleen Boris-Lawrie[1]

[1] HIV Center for RNA, Department of Veterinary Biosciences, Center for Retrovirus Research, Center for RNA Biology, Ohio State University, Columbus Ohio, USA; [2] Center for Retrovirus Research, College of Pharmacy, Ohio State University, Columbus Ohio, USA

RNA helicases are encoded by all eukaryotic and prokaryotic cells and a minority of viruses. They are active in all facets of cellular RNA biology and innate response to infection. The functional repertoire of RNA helicases is immense. Some promote viral replication, while others promote the antiviral state. We used proteomic approaches to interrogate the host RNA helicases that bind retroviral mRNA transcripts at the 5'-leader and investigated candidates for their biochemical and biophysical parameters of specificity and functional activity in HIV-1 replication. Herein, we present studies of DHX9/RNA helicase A (RHA) activity in HIV-1 propagation. RHA downregulation reduces the translation of all HIV-1 transcripts and is rescued by siRNA-resistant alleles. Coimmunopreciptiation and domain analysis demonstrated N-terminal residues of RHA specifically and selectively recognize structural features of the 5' RNA terminus corresponding to R U5 and PBS. The specific recognition of the cognate RNA tethers ATP-dependent activity that facilitates cap-dependent ribosome scanning and results in polyribosome accumulation on the viral open reading frames. Moreover, the recognition of the cognate RNA is necessary for incorporation of RHA into virions. The stoichiometry of ~2 RHA molecules per particle is proportional to the viral genomic RNA. RHA-deficient virions are less infectious, indicating an RNA-dependent role for RHA beyond translational activity in propagation of infectious HIV-1. While ATPase activity was necessary for viral RNA translation, nonenzymatic chaperone activity is sufficient to propagate infectious virions. RHA domain mutants have been studied for their ability to be incorporated into HIV-1 particles and their ability to rescue infectivity. Our results demonstrate two non-redundant functions of RHA are directed by cognate RNA, thus providing new insight into the molecular activity of host RNA helicases during infection.

55. GB Virus Type C E2 Protein Interferes with HIV-1 Gag Plasma Membrane Targeting Through Inducing ADP-Ribosylation Factors 1 Degradation

Chenliang Wang[1,2], Christine L. Timmons[1], Huanliang Liu[2*], Bindong Liu[1]

[1]Center for AIDS Health Disparities Research, Meharry Medical College, Nashville, Tennessee and [2]Institute of Gastroenterology and Institute of Human Virology, Sun Yat-sen University, Guangzhou, Guangdong, Peoples of Republic of China

GB virus type C (GBV-C) is a single-stranded positive-sense RNA virus. Not only is GBV-C nonpathogenic, infection with GBV-C has been linked to having a positive effect on the clinical outcome of HIV-1 infected patients. HIV-1 infected individuals who are coinfected with GBV-C presents with lower HIV-1 load, higher CD4+T-cell count, and longer survival rates compared to patient who are HIV-1 infected but GBV-C negative. We recently reported that the expression of GBV-C E2 inhibits HIV-1 assembly and release through interference with HIV-1 Gag plasma membrane targeting implicating a mechanism by which GBV-C inhibits HIV-1. In the current study we identified the cellular mechanism by which expression of GBV-C E2 inhibits HIV-1 Gag membrane targeting. We show that down-regulation of the ADP-Ribosylation Factors 1 (ARF1) plays a key role in this inhibitory process. ARF1 is one of many cellular factors that HIV-1 relies on for proper assembly and trafficking. Expression of glycosylated GBV-C E2 down regulates ARF1 expression without inhibiting ARF1 transcription. ARF1 degradation mediated through GBV-C E2 expression can be rescued by different proteasomal inhibitors (MG132, Bortezomib, LLnL), but not by Ubiquitin-Activating Enzyme (E1) inhibitor PYR-41. No ubiquitylated ARF1 was observed when we used an *in vivo* polyubiquitylation assay. Expression of exogenous ARF1 or proteasomal inhibitors compromised the inhibitory effect of GBV-C E2 on HIV-1 Gag processing. These finding indicate that GBV-C E2 impaired HIV-1 Gag targeting to the plasma membrane by inducing ARF1 degradation through an ubiquitin-independent proteasomal degradation pathway. Understanding the mechanism of which GBV-C E2 inhibits HIV-1 assembly and release will provide insights for novel anti-HIV/AIDS drug design.

56. The mRNA-Binding Protein YB1 Participates in the Assembly of a Murine Betaretrovirus

Darrin V. Bann, Andrea R. Beyer, and Leslie J. Parent

Departments of Medicine and Microbiology & Immunology, Penn State College of Medicine, Hershey, PA

The Gag protein of mouse mammary tumor virus (MMTV) orchestrates the assembly of complete, immature capsids in the cytoplasm of infected cells. The yeast Ty1 and Ty3 retrotransposons follow a morphogenetic pathway similar to MMTV and assemble virus-like particles in cytoplasmic ribonucleoprotein complexes called "retrosomes," which contain cellular mRNA binding proteins associated with stress granules (SGs) and processing bodies. These observations led us to conduct parallel experiments in MMTV to test whether sites of metazoan retrovirus immature capsid formation also contained SG proteins. We found that cytoplasmic sites of MMTV capsid assembly were distinct from SGs but colocalized with the SG-associated protein YB1, which was required for efficient virus production. Overexpression of YB1 and other SG-associated proteins, including G3BP1, induced the accumulation of Gag within SGs through an RNA-dependent mechanism. To test whether immature particle formation occurred within SGs, we expressed an MMTV Gag-mCherry fusion protein in living mouse mammary cells and used fluorescence recovery after photobleaching (FRAP) analysis to identify early, mid, and late phases of immature capsid assembly. Although we did not observe assembly of MMTV capsids within SGs, recovery of Gag-mCherry fluorescence after photobleaching was significantly reduced in SGs induced by YB1 overexpression compared to SGs induced by G3BP1 overexpression. These results suggest that Gag is stably bound to a factor within YB1-induced SGs, possibly YB1 itself. Together, these data suggest a model whereby YB1 recruits viral RNA into cytoplasmic RNPs where it is then bound by Gag for encapsidation into immature intracytoplasmic particles.

57. Characterization of HIV-1 Matrix Interactions

Ayna Alfadhli, Rachel Sloan, Isabel Cylinder, Sarah Gabriel, Claudia López, Henry McNett, and Eric Barklis

Vollum Institute and Department of Microbiology, Oregon Health and Science University, Portland, Oregon 97239

The matrix domain (MA) of the HIV-1 precursor Gag (PrGag) protein directs PrGag proteins to assembly sites at the plasma membrane by virtue of its affinity to the phospholipid, phosphatidylinositol-4,5-bisphosphate (PI(4,5)P(2)). Additionally, MA has been ascribed other functions such as RNA binding, and facilitating the incorporation of HIV-1 envelope (Env) proteins into virus particles. We have demonstrated that MA binds to RNA at a site that overlaps its PI(4,5)P(2) site, suggesting that RNA binding may protect MA from associating with inappropriate cellular membranes prior to PrGag delivery to the PM. Based on this, we have developed assays in which small molecule competitors to MA-RNA binding can be characterized, with the assumption that such compounds might interfere with essential MA functions and help elucidate additional features of MA binding. Following this approach, we have identified compounds that compete with RNA for MA binding. We also have identified MA residues involved in binding and found that they overlap the MA PI(4,5)P(2) and RNA sites. Cell culture studies demonstrated that these compounds inhibit HIV-1 replication but are associated with significant levels of toxicity. Nevertheless, these observations provide new insights into MA binding and pave the way for the development of antivirals that target the HIV-1 matrix domain.

58. Identification of a Matrix Mutation that Globally Rescues Env Incorporation Defects: Implications for Matrix Structure and Env Recruitment

Philip R Tedbury, Sherimay Ablan, Eric O Freed

Virus-Cell Interaction Section, HIV Drug Resistance Program, NCI-Frederick, Frederick, MD.

The matrix (MA) domain of HIV-1 Gag plays key roles in Gag-membrane targeting and Env incorporation into virions. Although a trimeric MA structure has been available since 1996, evidence for functional MA timers in either cells or virions has been elusive. The mechanism by which HIV-1 Env glycoproteins are recruited into virions likewise remains unclear. In the course of virus passaging experiments, we identified a point mutation in MA (62QR) that was able to globally rescue the Env incorporation defects imposed by an extensive panel of MA and Env (gp41) mutations. Mutagenesis of MA position 62 revealed that many residues were tolerated at that position but only Arg, and to a lesser extent Lys, were able to rescue an Env incorporation-defective MA mutant (12LE). Mapping the mutations onto the MA trimer structure revealed that the incorporation-defective mutations clustered at the tips of the trimer, around the perimeter of a gap in the MA lattice into which the cytoplasmic tail of gp41 could insert. By contrast, the rescue mutant 62QR was located near the center of the trimer. In fact, the side chain of Gln62 is within 5 Å of Ser66 and Thr69 in the neighboring MA molecule, suggesting that 62QR may effect rescue of Env incorporation via modification of MA trimer interactions rather than by promoting direct interaction between MA and gp41. To examine the importance of potential interactions between 62QR and Ser66 or Thr69 we mutated each residue to Ala and Arg. Subsequent analysis of virus infectivity revealed that Thr69 is required for rescue of 12LE by 62QR. In addition, the introduction of 66SR was able to rescue 12LE in the context of the wild-type Gln62, but blocked rescue when combined with 62QR, suggesting interference between the two positively charged side-chains. Our data strongly support the existence of MA trimers in the immature Gag lattice, based on the ability to predict likely interacting side-chains and the functional consequences of mutagenesis. Given the nature and location of MA mutations which block Env incorporation, it seems likely that the block is due to steric hindrance. Our data suggest this block can be relieved by modified interactions at the MA trimer interface, potentially altering the arrangement of the MA lattice. Furthermore, the importance of the trimer interface in rescuing Env incorporation defects suggests that the trimeric arrangement of MA may be a critical factor in permitting incorporation of Env into the Gag lattice. These results have significant implications for our understanding of Gag structure and Env packaging.

59. Gibbon Ape Leukemia virus Envelope (GaLV Env), an unexpected Vpu target.

Marc C. Johnson, Sanath K. Janaka, Tiffany M. Lucas, Terri D. Lyddon, Devon A. Gregory

Department of Molecular Microbiology and Immunology, University of Missouri-School of Medicine, Columbia, MO 65211

HIV-1 efficiently forms pseudotyped particles with most gammaretrovirus glycoproteins but is incompatible with the glycoprotein from GaLV. The HIV-1 accessory protein Vpu was found to be responsible for this incompatibility. In the presence of Vpu, GaLV Env is prevented from being incorporated into HIV-1 viral particles, eliminating particle infectivity. We have used this loss of infectivity as a robust and quantitative biological assay for Vpu activity.

Using a systematic mutagenesis scan, we determined the motif that makes GaLV Env sensitive to Vpu is INxxIxxVKxxVxRxK in the cytoplasmic tail domain (CTD). This region in the CTD of GaLV Env is predicted to form a helix. Mutations in the CTD that would break this helix abolish sensitivity to Vpu. This element bears striking similarity to sequences in the CD4 cytoplasmic tail. Using functional complementation we have demonstrated that Vpu can only modulate the GaLV Env CTD in the context of a CTD trimer.

We performed a comprehensive mutagenic scan of the cytoplasmic domain of Vpu in its native proviral context to identify features required for modulation of GaLV Env and BST-2/tetherin. We observed considerable overlap in the Vpu sequences required to modulate these two targets. We found that features in the cytoplasmic tail of Vpu, specifically within the cytoplasmic tail hinge region, were required for modulation of both tetherin and GaLV Env.

GaLV Env is synthesized as an 85 kDa precursor which is cleaved by a furin-like protease into mature 70 and 15 kDa products in a post-endoplasmic reticulum (ER) compartment. Vpu expression diminishes the level of mature Env in the cell, but does not affect levels of the 85 kDa precursor. Proteosomal inhibitors do not restore mature protein expression levels or infectivity, but expression of a dominant negative ubiquitin (K7R) partially restores infectivity. Lysosomal inhibitors restore mature protein expression, but do not restore infectivity. These data suggest that Vpu modulates GaLV Env by altering its trafficking in a ubiquitin-dependent fashion.

60. Vpu and Host Protein Complexes

Shujun Yuan[1], Martin Lynn[1], Wu Shenping[1], Shenheng Guan[2], Yifan Cheng[1], Robert M. Stroud[1]

[1]Department of Biochemistry and Biophysics, University of California San Francisco, San Francisco, CA; [2]Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA

Viral protein U (Vpu) plays a central role in HIV induced pathogenesis. It is the only HIV-1 accessory protein that contains a transmembrane domain. Vpu hijacks the host SCF (Skp1-Cul1-F-box protein) E3 ubiquitin ligase, leading to degradation of host proteins such as CD4 and facilitating virus particle release through interactions with tetherin. To understand how Vpu hijacks the SCF E3 ligase, we reconstituted and characterized the Vpu-E3 ligase protein complex that is consisted of Vpu cytoplasmic domain (VpuC), βTrCP-skp1, and Cul1-rbx1. Phosphorylated VpuC (pVpuC) forms a protein complex with purified βTrCP-skp1 and Cul1-rbx1. Phosphorylation of both Ser52 and Ser56 is essential for the formation of a stable pVpuC-βTrCP-skp1 complex, but phosphorylation of Ser52 is more critical than phosphorylation of Ser56 in forming the complex.

61. Analysis of HTLV-1 Particle Morphology and Gag Stoichiometry

Jose O. Maldonado[1,2], Iwen F. Grigsby[1,2], Sheng Cao[1,2], Wei Zhang[1,2], and Louis M. Mansky[1,2,3]

[1]Institute for Molecular Virology, [2]Department of Diagnostic and Biological Sciences, School of Dentistry, [3]Department of Microbiology, Medical School, University of Minnesota, Minneapolis, MN 55455

The Gag polyprotein is the main structural protein of retroviruses and is essential for the assembly and release of virus particles. In the absence of other retroviral proteins, Gag is sufficient for the production and release of viral-like particles (VLPs), which are structurally similar to immature infectious virions. Even with more tractable retroviral systems, the details for how Gag oligomerizes, trafficks to the plasma membrane, and drives the release of immature virus particles is poorly understood, particularly for the deltaretroviruses. We are currently focused on two areas of study: 1) analyze the morphology of the immature lattice formed in particles by the Gag polyprotein, and 2) study the determinants of Gag copy number per virus particles. Using cryogenic transmission electron microscopy (cryo-TEM), we have been analyzing the morphology of HTLV-1 VLPs. We have been investigating the average Gag copy number of HTLV-1 VLPs by scanning transmission electron microscopy (STEM). These fundamental studies of HTLV-1 assembly will lead to detailed information about these processes that will be useful for a better understanding of how these viruses replicate in cells. Such information may inform new therapeutic strategies.

62. Charged Residues in Surface-Exposed Capsid Loops Mediate Immature Retroviral Assembly and Maturation

Katrina J. Heyrana, Tam-Linh N. Nguyen, Maria C. Bewley, and Rebecca C. Craven

Department of Microbiology and Immunology, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033

The flexible loop (FL) of the retroviral capsid protein CA is a charge-rich region spanning the loops and helices between α4 and α7 of the CA N-terminal domain (residues 86-127 in Rous sarcoma virus CA NTD). This region of CA is thought to have essential functions in maintaining structural integrity of both immature and mature retroviruses, but the dual role is poorly understood. Structural studies suggest that, in the Gag shell of an immature retrovirus, adjacent FLs form a dimeric interface (Bharat *et. al., Nature* 2012). While no viral studies have tested the presence of this interface directly, mutations in the FL region result in Gag particle size dysregulation in RSV and prevent release or mature core formation of MLV, implying a role for the FL in early virus formation and egress. In the mature virus, the FL does not appear to make direct contacts but is a common point for cellular factor binding. In particular, TRIM5α studies indicate a role for the FL in mature capsid integrity, as TRIM5α -CA binding can result in accelerated core dissociation.

Based upon comparison of recent structural data with older genetic studies from our laboratory, we propose that the charges in the FL control both the formation of an immature FL-FL interface and the structural rearrangements that produce a stable mature core. To test these ideas, we undertook a mutagenesis screen of charged residues in this region of Rous sarcoma virus. A series of alanine and charge-swap mutations were tested for effects on virus infectivity and particle assembly. The charges at three positions, D87 and E99 (in the α4- α5 loop) and K107 (α5), proved to be especially critical for replication. Gag protein release and Gag processing patterns were minimally affected, but the size and shape of the released particles were strikingly disordered in the non-infectious mutants. These findings suggest a dysregulation of Gag-Gag interactions during immature particle assembly. Mutant CA proteins retained the ability to assemble *in vitro*, indicating that mature CA-CA interactions to form higher-order structures were preserved. These findings imply that electrostatic interactions at key positions in the FL region regulate optimal Gag packing during immature particle assembly. Modeling of the RSV CA protein places these charged residues within the Gag-Gag interface described by Bharat et al. (*Nature 2012*). Furthermore, the need to separate this highly charged interface to allow capsid maturation to proceed argues that proper management of electrostatic interactions on these surface loops is essential to multiple steps of the virus assembly and disassembly pathways.

63. Magic Angle Spinning NMR Reveals Sequence-Dependent Structural Plasticity, Dynamics, and the Spacer Peptide 1 Conformation in HIV-1 Capsid Protein Assemblies

Yun Han[1,2], Guangjin Hou[1,2], Manman Lu[1,2], Christopher L. Suiter[1,2], Jinwoo Ahn[2,3], In-Ja L. Byeon[2,3], Andrew S. Lipton[4], Sarah Burton[4], Ivan Hung[5], Peter Gor'kov[5], Zhehong Gan[5], William Brey[5], David Rice[6], Angela M. Gronenborn[2,3], and Tatyana Polenova[1,2]

[1]Department of Chemistry and Biochemistry, University of Delaware, Newark, DE; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA; [4]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA; [5]National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL; [6]Agilent Technologies, Inc., 5301 Stevens Creek Boulevard, Santa Clara, CA

Maturation of the HIV-1 virus into an infectious virion requires cleavage of the Gag polyprotein into its constituent domains and formation of a conical capsid core that encloses viral RNA and a small complement of proteins for replication. The final step of this process is the cleavage of the SP1 peptide from the CA-SP1 maturation intermediate, which triggers the condensation of the CA protein into a conical capsid. The mechanism of this step, including the conformation of the SP1 peptide in CA-SP1, is under debate.

In this work, we examine the tubular assemblies of CA and the CA-SP1 maturation intermediate using magic angle spinning NMR spectroscopy. At the magnetic fields of 19.9 T and above, tubular CA and CA-SP1 assemblies yield outstanding-quality 2D and 3D MAS NMR spectra, which are amenable to resonance assignments and detailed structural characterization. Dipolar- and scalar-based correlation experiments unequivocally indicate that SP1 peptide is in a random coil conformation and mobile in the assembled CA-SP1.

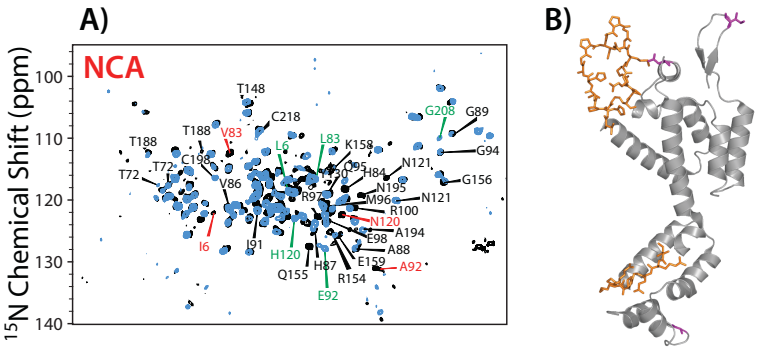


Figure 1. A) The superposition of the NCA spectra of CA NL4-3 A92E (blue) and CA HXB-2 black. The red and green labels represent signals from the distinct residues in the NL4-3 A92E and HXB-2 variants, respectively. B) A view of the 3D structure of CA (HXB2 variant, PDB file 3NTE). Colored magenta are the residues that vary between the two constructs; shown in orange are the residues that are identical in the two variants but whose chemical shifts are perturbed.

Analysis of two sequence variants reveals that, remarkably, the conformation of the SP1 tail, of the functionally important CypA loop, and of the loop preceding helix 8 are sequence dependent and modulated by the residue variations at distal sites. These findings make us revisit the question of the role of SP1 as a conformational switch in the maturation process and establish sequence-dependent conformational plasticity in CA.

References

1. Gottlinger HG (2001) The HIV-1 assembly machine. *Aids* 15:S13-S20.
2. Sundquist WI & Krausslich HG (2012) HIV-1 Assembly, Budding, and Maturation. *Csh Perspect Med* 2(7).
3. Du S., *et al.* (2011) Structure of the HIV-1 Full-Length Capsid Protein in a Conformationally Trapped Unassembled State Induced by Small-Molecule Binding. *J Mol Biol* 406(3): 371-386.

64. RNA-Mediated Regulation of p15NC Processing: Revisited

Marc Potempa[1], Sook-Kyung Lee[2], and Ronald Swanstrom[1,2]

[1]Department of Microbiology and Immunology, [2]Department of Biochemistry and Biophysics, and the UNC Center for AIDS Research, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

The HIV-1 protease must cleave the structural polyprotein Gag into its constituent parts in an ordered, highly regulated series of events. In this process, p15NC (comprised of nucleocapsid (NC), spacer peptide 2 (SP2), and p6) is separated from a membrane-bound portion of Gag, enabling the ribonucleoprotein core of the virion to begin condensing. In a subsequent step, the p6 region of p15NC must be removed. Previous reports have identified RNA as facilitating the cleavage event between SP2 and p6. To investigate the mechanistic basis of the RNA-mediated regulation of p15NC processing, we utilized an *in vitro* two-substrate proteolysis system comprised of the p15NC protein and a substrate that recapitulates the MA/CA cleavage event as an internal control. While we have observed RNA-mediated acceleration of SP2/p6 processing at pH >6.0, the relative magnitude of the acceleration is indistinguishable from the increased rate of MA/CA cleavage upon inclusion of RNA in the reaction system. However, at pH <6.0, cleavage of p15NC demonstrates a significant increase in processing rate relative to the MA/CA protein. As the pH of the HIV-1 virion is currently believed to be near physiological pH, our preliminary results call into question the biological relevance of RNA-mediated enhancement of p15NC processing beyond a general rate enhancement of proteolysis in the presence of RNA.

65. Electron Cryo-Tomography Studies of Maturing HIV-1 Viral Particles

[1]Cora L. Woodward, [1,2]Grant J. Jensen

[1]Division of Biology and [2]HHMI, California Institute of Technology, 1200 E California Blvd, Pasadena, CA 91125, USA

Maturation of the HIV-1 viral particle begins with the ordered proteolytic disassembly of the immature Gag lattice by the viral enzyme protease. Subsequent reassembly of the processed Gag constituent, CA, produces an infectious particle characterized by a cone-shaped core that encloses the viral RNA genome. Although the sequential proteolytic cleavage steps leading to maturation are well described, the structural intermediates concomitant to the maturation process remain poorly understood. Computational simulations that model the formation of the mature HIV-1 core as the non-equilibrium growth of an elastic sheet predict that core assembly involves the union in space of two edges of a curling sheet[1]. To determine if CA "sheets" are formed as structural intermediates during maturation, we have carried out electron cryo-tomography (ECT) studies on viral particles cryo-preserved next to infected cells. This *in vivo* population of viral particles is asynchronous with respect to the maturation process, and, therefore, mature, immature and intermediate maturation forms are represented. Our analysis of the *in vivo* particles suggests the presence of sheet-like structures in some of the particles. We also observe a correlation between RNP localization and mature conical-shaped core morphology. On-going work is aimed at *i*) categorizing the maturation intermediate structures observed by ECT, *ii*) determining where the structures fit in the process of maturation, and *iii*) the development and application of automated segmentation tools to facilitate analysis of the 3D density maps of the maturing viral particles.

1. Yu Z, et al., JMB 22013 Jan 9;425(1):112-23

66. Revealing the Structure of the HIV-1 Capsid

Juan R. Perilla[1], Gongpu Zhao[2,3], Peijun Zhang[2,3], and Klaus Schulten[1]

[1]Department of Physics and Beckman Institute, University of Illinois Urbana-Champaign, 61801; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15260; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15260

We recently derived the structure of the helical CA lattice, by Molecular Dynamics Flexible Fitting (MDFF) of high resolution structures (hexamers and dimers) into the cryo-electron microscopy density of cylindrical CA assemblies *in vitro* [1]. The MDFF derived hexameric lattice in its native, curved conformation was deposited to the protein data bank (PDB code 3J34) as a hexamer of hexamers (HOH). The MDDF-derived HOH was then used to model the cylindrical HIV capsid assembly. Modeling of a CA pentamer surrounded by five CA hexamers (known as pentamer of hexamers (POH))[1], showed that the CA hexamers readily relaxed into a more steeply curved conformation. The differences between the POH and HOH structures revealed key interactions at the three-fold symmetry axis that modulate the bite angle between neighboring oligomers. Based on cryo-electron tomography and by analogy with fullerenes, along with the POH and HOH models, the structure of the entire HIV-1 capsid was derived resulting in two representative structures (PDB codes 3J3Q and 3J3Y) [1]. In this poster we present the methodology employed to build the HIV-1 capsids from its building blocks.

1. Mature HIV-1 capsid structure by cryo-electron microscopy and all-atom molecular dynamics. Gongpu Zhao* , Juan R. Perilla*, Ernest L. Yufenyuy*, Xin Meng, Bo Chen, Jiying Ning, Jinwoo Ahn, Angela Gronenborn, Klaus Schulten, Christopher Aiken, and Peijun Zhang. *Nature*, 2013 497, p. 643-646

67. Probing Dynamics in HIV-1 Capsid Protein Assemblies by a Hybrid Solid-State NMR / Molecular Dynamics Approach

Guangjin Hou[1,2], Huilan Zhang[1,2], Christopher L. Suiter[1,2], Yun Han[1,2], Suvrajit Maji[3], Judith Klein[3], Christopher J. Langmead[3], Jinwoo Ahn[2,4], In-Ja L. Byeon[2,4], Angela M. Gronenborn[2,4], Tatyana Polenova[1,2]

[1]Department of Chemistry and Biochemistry, University of Delaware, Newark; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, 1051 Biomedical Science Tower 3, 3501 Fifth Ave., Pittsburgh; [3]Computer Science Department, Carnegie Mellon University, Gates Hillman Center, 5000 Forbes Avenue, Pittsburgh; [4]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh

The 26.6 kDa capsid protein (CA) of human immunodeficiency virus 1 (HIV-1) assembles into a cone-like structure, enclosing the viral RNA genome and a small complement of proteins during viral maturation. The host protein Cyclophilin A (Cyp A) binds CA and facilitates HIV-1 replication by an unknown mechanism. The CA-CypA interaction has been known to increase HIV-1 susceptibility to restriction by TRIM5a. In order to elucidate the mechanisms leading to structural polymorphism in CA capsids, we conducted a hybrid solid-state NMR / molecular dynamics (MD) study of CA protein, focusing on the backbone motions occurring on the timescales of 10^{-9}~10^{-6} s. Ultra-high resolution NMR spectra obtained from the tubular CA assemblies at high magnetic fields permit to determine the structure and dynamics at atomic level. To this end, we have performed residue-resolved ^{1}H-^{15}N dipolar and ^{15}N CSA 3D NMR experiments on CA assemblies of two different sequence variants. The backbone ^{1}H-^{15}N dipolar order and ^{15}N CSA parameters recorded with RN-symmetry sequences indicate unusually high mobility in the CypA-binding loop region belonging to the N-terminal domain of CA (see Figure 1). Experimental results reveal that the backbone mobility depends significantly on the primary CA sequence. All-atom molecular dynamics calculations were performed on free CA, yielding the motional trajectories for every residue in a protein. Dipolar order parameters extracted from the 100-ns MD trajectories are in excellent agreement with the experimental results. The hybrid MAS NMR/MD approach established in this work is thus a powerful strategy for atomic-level analysis of dynamics in HIV-1 protein assemblies.

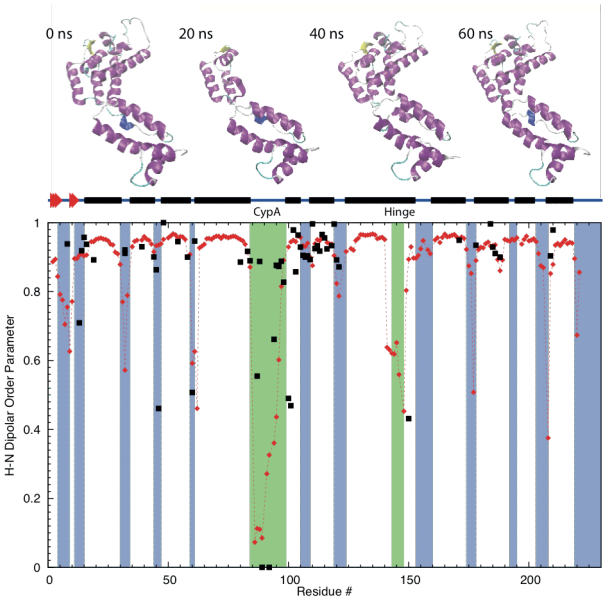


Figure 1. (Top): Snapshots of MD trajectories for CA. (Bottom) Plots of backbone H-N dipolar order parameters determined experimentally for CA tubular assemblies by MAS NMR (black) and extracted from MD calculations of CA (red), plotted vs. the residue number.

This work was supported by the National Institutes of Health (NIH Grants R01GM085306, P50GM082251, and P30GM103519 from NIGMS, and P30RR031160 from NCRR). We acknowledge the support of the National Science Foundation (NSF Grant CHE0959496) for the acquisition of the 850 MHz NMR spectrometer at the University of Delaware.

68. Structure of the Mature HIV-1 Capsid

Gongpu Zhao[1,2], Juan R. Perilla[3], Ernest L. Yufenyuy[2,4], Xin Meng[1,2], Jiying Ning[1,2], Jinwoo Ahn[1,2], Angela M. Gronenborn[1,2], Klaus Schulten[3], Christopher Aiken[2,4] and Peijun Zhang[1,2]

[1]Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA; [2]Pittsburgh Center for HIV Protein Interactions, Pittsburgh, PA 15260, USA; [3]Department of Physics and Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA; [4]Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

The mature human immunodeficiency virus 1 (HIV-1) contain conical capsids that enclose the viral RNA genome, enzymes and accessory proteins. HIV-1 capsid is comprised of capsid protein (CA), which contains two structural domains that are connected by a flexible linker. Structures of hexamic and pentameric CA assembly units were determined to atomic level. But the atomic structure of whole HIV-1 capsid is still lacking. Here, we present a cryo-EM structure of CA tubular assembly at 8 Å resolution and a cryo-electron tomography (cryo-ET) structure of native HIV-1 core. The structure of the tubular assembly reveals, at the three-fold interface, a three-helix bundle that exhibits critical hydrophobic interactions. Mutagenesis studies confirmed that hydrophobic residues in the center of the three-helix bundle are critical for capsid assembly and stability, and for viral infectivity. The cryo-EM structures permitted unambiguous modeling by large-scale molecular dynamic (MD) simulation, resulting in all-atom models for the hexamer-of-hexamer (HOH) and pentamer-of-hexamer (POH) elements of spheroidal capsids. The POH structure has revealed that incorporation of pentamers results in tighter trimer contacts and induces acute surface curvature. Based on the POH, HOH and cryo-ET structures, we have generated a realistic all-atom model of complete HIV-1 capsid by unconstrained, 100ns, 64 million atoms MD simulation.

69. Electron Tomography of HIV-1 Infection in Gut-Associated Lymphoid Tissue

Mark S. Ladinsky[1], Collin Kieffer[1], Gregory Olson[3], Douglas S. Kwon[3] and Pamela J. Bjorkman[1,2]

[1]Division of Biology 114-96 and [2]Howard Hughes Medical Institute, California Institute of Technology, 1200 East California Boulevard, Pasadena, CA 91125; [3]Ragon Institute of MGH, MIT and Harvard, 400 Technology Square, Cambridge, MA 02139;

Critical aspects of HIV-1 infection occur in mucosal tissues, particularly in the gut, which contains large numbers of HIV-1 target cells that are depleted early in infection. We used electron tomography (ET) to image HIV-1 in gut-associated lymphoid tissue (GALT) of HIV-1–infected humanized mice, the first three-dimensional ultrastructural examination of HIV-1 infection in vivo. The resolution and preservation quality of reconstructed tissue volumes was comparable to cryoET analyses of purified frozen hydrated virions and infected cultured cells. We localized dense regions of HIV-1 infected cells and virions to intestinal crypts, imaged HIV-1 transmission via virological synapses and intercellular pools of mature and immature virions, and resolved details of budding virions, including host-encoded scission machinery. Three-dimensional imaging of an active infection provided evidence of synchronous virus release and rapid maturation, revealed differences between cultured cell and tissue infection models, and furthered the ultrastructural understanding of HIV-1 transmission in mucosal tissue.

70. Capturing Enveloped Viruses on Affinity Grids for Downstream Cryo-Electron Tomography Applications

Gabriella Kiss[1], Xuemin Chen[1], Jens M. Holl[1], Melinda A. Brindley[1], Patricia Campbell[2], Lauren A. Byrd-Leotis[2], John Steel[2], David Steinhauer[2], Claudio L. Afonso[3], Richard K. Plemper[1], Paul Spearman[1], Deborah F. Kelly[4], and Elizabeth R. Wright[1]

[1]Division of Pediatric Infectious Diseases. Department of Pediatrics. Emory University School of Medicine. Children's Healthcare of Atlanta. Atlanta, GA 30322; [2]Department of Microbiology and Immunology. Emory University School of Medicine. GA 30322; [3]USDA, ARS, Southeast Poultry Research Laboratory, Athens, Georgia, USA; [4]Virginia Tech Carilion Research Institute, Roanoke, VA 24016

Enveloped viruses are a large population of known viruses that are human and animal pathogens. Therefore, studies to determine the overall architecture and fine ultrastructure of enveloped viruses are essential. Due to the processes associated with virus assembly and budding many of them exhibit varying degrees of structural irregularity or pleiomorphism. This structural variability can express itself as slight alterations to the size and internal architecture of the virus, such as with members of the Retroviridae family, to significant variations in the size and shape of the virus, as observed with members of the Orthomyxoviridae and Paramyxoviridae families.

Owing to their pleomorphic nature, cryo-electron microscopy (cryo-EM) and cryo-electron tomography (cryo-ET) are the most suitable methods for structural analyses. Both techniques involve the vitrification of highly purified and concentrated viral samples onto EM grids. Unfortunately, the structural heterogeneity of enveloped viruses negatively impacts the success of standard viral purification methods used for the production of highly concentrated and purified samples. Enveloped virus purification presents other challenges, specifically, the inclusion of host-membrane derived vesicles, the complete destruction of the viruses, or the disruption of the internal architecture of individual virus particles. Purification methods commonly involve chemical precipitation (PEG) and high-density gradients (sucrose or glycerol) for the separation of viruses from cell debris. However, these steps can alter virus structure, impact the titer, as well as artificially select for one subtype of particle morphologies.

Recently, "Monolayer Purification" and "Affinity Grid" methods were introduced to the EM field in order to apply His-tagged protein purification methods directly to the EM grid. Affinity grids are EM grids coated with a lipid layer that contains a large percentage of non-functionalized lipids combined with a variable percentage of Ni-NTA (Nickel-nitrilotriacetic acid) moiety lipids. The Ni-NTA active group binds directly to His-tagged proteins. Using His-tagged Protein A and a protein-specific antibody enables this technique to be used on non-His-tagged targets. This approach has been applied to the structural analysis of the ribosome from crude cell extracts, entire RNA processing pathways, and the development of an *in situ* biological TEM imaging platform.

Here we illustrate the application of affinity grid technologies for the purification and capture of pleiomorphic-enveloped viruses directly to EM grids for both conventional TEM and cryo-EM/cryo-ET studies. We examined affinity grids for the selective capture of human immunodeficiency virus (HIV) virus-like particles (VLPs), measles virus (MeV), influenza A, and Newcastle disease virus (NDV). The application of affinity grid methods may prove essential for the gentle and selective purification of enveloped viruses directly onto EM grids for ultrastructural analysis and provide novel prospects for imaging viruses that have been historically difficult to produce and purify by conventional methods.

71. Optical Trapping and Sorting of the Human Immunodeficiency Viruses at Single-Molecule Level

Yuanjie Pang, Hanna Song, Jin H. Kim and Wei Cheng*

*Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, 428 Church Street, Ann Arbor, MI 48109, USA. *Correspondence: chengwe@umich.edu*

Optical tweezers (OTs) use the momentum of photons to trap and manipulate microscopic objects contact-free in three dimensions. Although this technique has been widely used in biology to study molecular motors, biopolymers, cytoskeletons, and for sorting of microparticles, its application in pathogenic viruses has been very limited largely due to the small size of these particles. Using an improved OTs instrument that can simultaneously resolve two-photon fluorescence (TPF) at single-molecule level, here we show that individual human immunodeficiency virus type 1 (HIV-1) internally tagged with green fluorescent proteins (GFP) can be optically trapped and manipulated in culture media. The hydrodynamic radius (r_{hyd}) for each trapped virion can be directly measured, which yields r_{hyd} of 78 ± 15 nm, with a majority of the virions displaying slightly elongated shape. The wide intensity distribution of TPF from individual virions interrogated by the optical trap reveals that each HIV-1 incorporates a random number of Vpr molecules fused to GFP, with a range of 3-128 molecules per virion. By using fluorescent-labeled monoclonal antibodies specific for HIV-1 envelope glycoprotein gp120, we measured the envelope spike protein content for single virions in culture media. The quantity of spike proteins per virion varies over one order of magnitude even within the same batch of viruses, which implies substantial heterogeneity of these virions in transmission and infection at single-particle level. Analogous to flow cytometry for cells, this fluid-based technique may allow detection and multi-parameter analysis of viruses and other nanoparticles within heterogeneous populations at single-particle and single-molecule resolution (Supported by NIH 1DP2OD008693-01; WC).

72. Deep Sequencing of Protease Inhibitor Resistant HIV Reveals Patterns of Mutations in Gag

Max W. Chang[1], Jinyun Yuan[1], Glenn Oliveira[2], Samuel Levy[2], Jason F. Okulicz[3,4], and Bruce E. Torbett[1]

[1]Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA; [2]Scripps Genomic Medicine, Scripps Translational Science Institute, La Jolla, CA; [3]Infectious Disease Clinical Research Program, Uniformed Services University of the Health Sciences, Bethesda, MD; [4]Infectious Disease Service, San Antonio Military Medical Center, San Antonio TX

The development of resistance against HIV protease inhibitors has contributed to the persistence of the HIV/AIDS epidemic. While the role of drug resistance mutations in protease have been studied comprehensively, mutations in its substrate, Gag, have not been extensively cataloged. Using next-generation sequencing technologies, we sequenced viral samples from 96 patients who had been treated with therapies that included protease inhibitors.

Serum or plasma samples were obtained when therapy failed to adequately suppress viral replication (generally 1,000 copies/mL), allowing multiple samples to be taken for some patients. Following extraction of viral RNA from these patient samples, one-step RT-PCR was used to generate two 1-kb amplicons that spanned HIV Gag and protease. The RT-PCR products were sequenced using an Illumina HiSeq, producing an average of 4.3 million reads of 100 bp per sample. Overall, >90% of these reads were mapped to a Consensus B reference sequence, yielding more than 200,000-fold sequencing coverage on average.

Analysis of these sequences indicates the presence of known primary and accessory protease drug resistance mutations in many of the patients, as well as a low frequency of mutations in Gag cleavage sites. Other mutations with unknown effects were found throughout the Gag domains. Work is ongoing to correlate the presence of these Gag mutations to later protease inhibitor therapy outcomes.

73. Understanding the Function of Drug Resistance Mutations in the HIV Gag Polyprotein

Divita Garg, Sebastian Breuer, Bruce E. Torbett

The Scripps Reseach Institute, La Jolla, CA

Mutations in HIV-protease result in development of resistance against the known protease inhibitors. To compensate for the deleterious effect of protease mutations on Gag binding and processing, mutations can arise in the Gag. These mutations are found in the protease cleavage sites (CS) as well as in distal regions of Gag. Although the development of the compensatory mutations in Gag has been widely observed and studied in viruses, the mechanism of compensation is not well understood. This is partly caused by the inherent structural flexibility of the Gag domains and CS, which make it difficult to study the interactions of the protein with the protease. We are using our in-house CE-CBA assay to understand the effect of the Gag mutations on protease cleavage. Our data show that the cleavage site mutations A431V and L449F and distal site mutation T456F enhance the catalytic efficiency of the protease cleavage thereby contributing towards the observed resistance to protease inhibitors.

74. Selection for Resistance to Potent New HIV-1 Protease Inhibitors and Analyzed Using Deep Sequencing

Sook-Kyung Lee[1], Shuntai Zhou[1], Celia Schiffer[2], and Ronald Swanstrom[1]

[1]Department of Biochemistry and Biophysics, and the UNC Center for AIDS Research, University of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts, Worcester, MA

We conducted in vitro selections to each of 10 potent HIV-1 PIs (UMASS-1, 2, 3, 4, 5, 6, 7, 8, 9, and 10), which are derivatives of Darunavir (DRV), using a mixture of 26 PI-resistant HIV-1 variants. Each variant contains a single PI-resistance associated amino acid substitution in protease. Mutations in the protease region of the viral populations at the early two time points were analyzed using Primer ID based deep sequencing technology and compared with mutations detected by bulk sequencing. The mutations, I84V, A71T, and L63P, were observed frequently by both bulk and deep sequencing early during selection. These mutations were followed by mutations, L10F, G16E, M36I, M46I, I50V, V82I/F, and I93L. The minor variants frequently detected by only deep sequencing include L10I, K43R/E, P44T, M46L, G49E, R87K, and F99L. These mutations, except for L10I and M46L, have not been reported as PI-associated resistance mutations. The mutations selected by DRV at time point 2 were I13V, G16E, V32I, L33F, K45I, M46I, G49E, V82F, and I84V. At the final passage (p61), only two drugs (UMASS-2 and 6) reached drug concentration higher than 1 μM and the drug concentrations of other drugs were in the nanomolar range. For two of the inhibitors (UMASS-3 and -10), increase in the drug concentration was not successful beyond drug concentration in the subnanomolar range suggesting that these drugs have a higher genetic barrier to development of HIV-1 resistance than has DRV. Currently, mutations in the protease region of the viral population at later time points are further analyzed by deep and bulk sequencing, and mutations selected in the *gag* and *pol* regions will be discussed.

75. Differential Flap Dynamics in Wild-Type and a Drug Resistant Variant of HIV-1 Protease Detected by NMR Relaxation and MD Simulations

Rieko Ishima[1], Wazo Myint[1], Yufeng Cai[2], Nese Kurt Yilmaz[2], Celia A. Schiffer[2]

[1]Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA15260; [2]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA01605.

Nuclear Magnetic Resonance (NMR) relaxation provides site-specific information on protein internal dynamics associated with configurational energetics and conformational equilibrium.[1] Since NMR techniques to study structure and dynamics of biomolecules have tremendously improved in the last decade, experiments that previously did not yield sufficiently sensitive data can now provide new insights into biomolecular mechanisms. To characterize protein backbone dynamics, a set of ^{15}N longitudinal relaxation rate (R_1), ^{15}N transverse relaxation rate (R_2), and the heteronuclear ^{15}N-{^1H} NOE experiments are routinely applied. Similarly, ^{15}N Carr-Purcell-Meiboom-Gill (CPMG) relaxation dispersion experiment is frequently employed to detect protein backbone conformational exchange on milli-micro second time scale. However, to detect relatively small but relevant changes in dynamics, such as those between wild-type and mutant proteins, further improvement of the analysis method is necessary.[2] Using our newly optimized method for analyzing NMR relaxation data, we recently characterized backbone dynamics of a multi-drug-resistant variant of HIV-1 protease with a combination of mutations at the edge of the active site, within the active site, and in the flaps (L10I, G48V, I54V, V82A),[3,4] and compared the results with those for the wild-type protease. Molecular Dynamics (MD) simulations on wild-type and mutant protease were consistent with and helped explain the NMR observations. The impact of the mutations on enzyme dynamics, especially in the flap regions, was elucidated in detail by a combination of MD simulations and NMR relaxation results.[2]

References

1. Ishima R. (2012) Top Curr Chem, 326, 99-122.
2. Cai, Y, Yilmaz, NK, Myint,W., Ishima R. & Schiffer C. (2012) J Chem. Theory Comput, 8, 3452-62.
3. King NM, Prabu-Jeyabalan M, Bandaranayake RM, Nalam MN, Nalivaika EA, Özen A, Haliloğlu T, Yilmaz NK, Schiffer CA. (2012) ACS Chem Biol, 7, 1536-46.
4. Foulkes-Murzycki JE, Rosi C, Kurt Yilmaz N, Shafer RW, Schiffer CA. (2013) ACS Chem Biol, 8, 513-8.

76. Protein Stability, Drug Resistance, and Fitness Landscapes of HIV-1 Protease

Omar Haq, William Flynn, R. S. K. Vijayan, Anthony Felts, Ronald M. Levy

Department of Chemistry and Chemical Biology, Rutgers University

Proteins have a narrow window of stability that leads to optimal functionality. HIV-1 protease mutates at a rapid rate to resist inhibitors; however, many of the primary resistance mutations are destabilizing. Secondary correlated mutations compensate for the loss of stability inflicted by primary mutations. We have characterized correlated charged mutations in HIV-1 protease which preserve the stability of the fold. We have also developed a coarse-grained electrostatic model based on the implicit generalized Born solvation model, AGBNP, which captures the effect of making mutations that change the formal charge of the wild type residue. Statistical modeling indicates that the network of correlated electrostatic mutations has a simple topology and has evolved to minimize frustrated interactions. We are extending the characterization of correlated mutations to a more detailed atomic model including nonpolar and polar residues using a larger library of states. The neutral state may reflect differences in polarity, aromaticity and size. Energetic calculations will require using a more complete and detailed all-atom force field than the coarse-grained version of AGBNP used previously. We will achieve that using a combination of Rosetta and IMPACT for the structure-based modeling. Backbone and side chain motions can be captured using a combination of X-ray crystallographic structures. The all-atom force field will need to capture nonelectrostatic effects such as side chain packing and changes in hydrophobicity at a particular site. A double mutant thermodynamic cycle approach can be used to identify long-range correlated mutations which stabilize the protease fold. We intend to investigate the role of correlated mutations in the acquisition of drug resistance.

1. Haq, O.; Levy, R. M., Morozov, A. V.; Andrec, M. Pairwise and higher-order correlations among drug-resistant mutations in HIV-1 subtype B protease. *BMC Bioinformatics,* 2009, *10,* S10.
2. Haq, O.; Andrec, M.; Morozov, A. V.; Levy, R. M. Correlated electrostatic mutations provide a reservoir of stability in HIV protease. *PLOS Comput. Biol.,* 2012, *8,* e1002675.
3. Wickstrom, L.; Gallicchio, E.; Levy, R. M. The linear interaction energy method for the prediction of protein stability changes upon mutation. *Proteins: Struct. Funct. Bioinform.,* 2012, *80,* 111-125.

77. Extreme Entropy-Enthalpy Compensation Due to Cooperative Mutations in the Flap Region of HIV-1 Protease

Neşe Kurt-Yılmaz, Nancy M. King, Moses Prabu-Jeyabalan, Jennifer E. Foulkes-Murzycki, Rajintha M. Bandaranayake, Madhavi N. L. Nalam, Ellen A. Nalivaika, and Celia A. Schiffer

Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA 01605

The development of HIV-1 protease inhibitors (PIs) has been the historic paradigm of rational structure-based drug design, where structural and thermodynamic analyses have led to the discovery of potent inhibitors. However, under the selective pressure of therapy, multiple mutations accumulate in protease to confer drug resistance and render PIs ineffective. Characterizing the interdependence of such mutations in altering structural and inhibitor binding energetics of the protease is crucial to the development of novel and robust inhibitors.

In the current study, profound changes were observed in the inhibitor binding thermodynamics of a drug-resistant variant compared to wild-type HIV-1 protease. The drug resistant variant Flap+$_{I54V}$ (L10I/G48V/I54V/V82A) displays extreme entropy–enthalpy compensation (5-15 kcal/mol) relative to wild-type enzyme irrespective of the PI bound. The co-crystal structures of Flap+$_{I54V}$ with four PIs were determined and compared with complexes of both the wild-type protease and another drug-resistant variant that does not exhibit this energetic compensation. Structural changes conserved across mutant complexes, which are the most pronounced for the flaps covering the active site, likely contribute to the observed unusual thermodynamics. Interestingly, a very similar variant with an Ala at position 54, Flap+$_{I54A}$, does not display such extreme entropy-enthalpy compensation. Additionally, single flap mutants (G48V and I54V) reveal that the effect of mutations are not simply additive but highly cooperative in altering the thermodynamics of inhibitor binding.

The finding that a particular combination of drug-resistant mutations exerts cooperative effects to profoundly modulate the relative thermodynamic properties of a therapeutic target independent of the inhibitor presents a new challenge for rational drug design.

King, NM et al., *ACS Chemical Biology*, 2012, 7 (9), 1536–1546.
Foulkes-Murzycki, JE et al., *ACS Chemical Biology*, 2013, 8 (3), 513–518.

78. Advancing the Discovery of Allosteric Inhibitors of HIV Protease Using Virtual Screens on FightAIDS@Home

Alexander L. Perryman, Ying-Chuan Lin, Subash Velaparthi, Max Chang, Michael Baksh, Stefano Forli, Srinivas Reddy Chirapu, Theresa Sample, David Goodsell, C. David Stout, Valery Fokin, M.G. Finn, Bruce Torbett, John Elder, and Arthur J. Olson

The Scripps Research Institute, La Jolla, CA, USA.

To help defeat the multi-drug-resistant mutants of HIV that are constantly evolving, new types of drugs with novel mechanisms of action are needed. Since combinations that include two different classes of inhibitors that act on the same enzyme have been shown to decrease the probability of eliciting new drug-resistant mutants, we are attempting to advance the discovery of allosteric inhibitors of HIV protease (PR) by performing virtual screens (VS) on FightAIDS@Home (an internet-distributed computing project that is part of IBM's "World Community Grid"). The Asinex library of ~360,000 compounds was docked against the "4D9 exo site" on the sides of PR, using AutoDock 4.2.[1,2] Different sets of energetic and interaction-based filters were tested using in-house scripts from "Raccoon 2".[3] The final set of filters harvested 160 compounds, whose binding modes were visually inspected. 4 of the 34 candidates we ordered caused a significant thermal shift in differential scanning fluorimetry assays (DSF) performed by the Torbett lab, and 2 of these 4 DSF hits displayed low micro-M affinity in backscattering interferometry assays (BSI) performed by the Finn and Torbett labs. All 4 fragment hits also displayed weak inhibition of PR activity in the standard FRET-based assay in the Elder lab. To test the Structure-Activity-Relationships displayed by these hits, the Fokin lab synthesized 8 derivatives, which were then assayed by the Elder lab. The structures of the original 4 hits were also used as queries to search the ZINC server[4] for somewhat similar compounds, and a focused library of 2,574 compounds was created and used in a new VS. 44 new candidate compounds were purchased and then assayed by the Elder lab. 7 of these new candidates also inhibited PR activity, and 6 of them were more potent than the original 4 parent compounds. These 11 hits represent the first compounds identified in virtual screens against the 4D9 exo site that displayed inhibition of HIV PR activity.

References:

1. Morris, G.M., Huey, R., Lindstrom, W., Sanner, M.F., Belew, R.K., Goodsell, D.S., and Olson, A.J. (2009) AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J. Comput. Chem., 30(16), 2785-2791.
2. Perryman, A.L., Zhang, Q., Soutter, H.H., Rosenfeld, R., McRee, D.E., Olson, A.J., Elder, J.E., and Stout, C.D. (2010) Fragment-based screen against HIV protease. Chemical Biol. & Drug Design, 75 (3), 257-268.
3. Forli, S., Olson, A.J., and Sanner, M.F. http://autodock.scripps.edu/resources/raccoon2
4. Irwin, J.J., Sterling, T., Mysinger, M.M., Bolstand, E.S., and Coleman, R.G. (2012) ZINC: A free tool to discover chemistry for biology. J. Chem. Inf. Model., 52(7), 1757-1768.

79. Using Absolute Binding Free Energy Methods to Identify True Binders to Allosteric Sites on HIV Protease

Nanjie Deng, Peng He, Alex Perryman, Stefano Forli, Emilio Gallicchio, Art Olson, Ronald Levy

Department of Chemistry, Rutgers University, Piscataway, New Jersey; The Scripps Research Institute, La Jolla, California

Absolute binding free energy methods in principle provide a rigorous route to the prediction of ligand binding affinities. Although these methods are currently limited in their ability to predict rank ordering for binders, they can be fruitfully employed to separate binders from non-binders. HIV-1 protease has been a major drug target in developing anti-viral therapy against AIDS. Using docking and experimental assays, the Stout and Olson labs recently discovered a new allosteric binding site located on top of the HIV-PR flaps.[1] We have applied absolute binding free energy methods in both implicit and explicit solvent to distinguish true binders from false positives among a set of ligands that dock favorably to the allosteric site.

Top ranked protein-ligand complexes from AutoDock were used as the starting point for free energy calculations. Two methods, the Binding Energy Analysis method (BEDAM) developed by our lab,[2] and the Double Decoupling Method (DDM) were employed. Absolute binding free energies are computed for 21 ligands, including 2 actives, 6 possible binders, and 13 false positives. The fact that the 13 false positives received the most favorable scores by docking increases the challenge for free energy methods. Our calculations using both BEDAM and DDM correctly identified 70% of the false positives as well as the two actives. Three out of six possible binders are predicted to bind, a finding that requires further confirmation by NMR screening with site labeling. The ROC curves calculated using the two methods show similar, substantial enrichment.

The free energy simulations revealed important structural transitions induced by the ligand binding to the allosteric site. During the course of the DDM calculation for the active ligand 1F1N, the intramolecular hydrogen bond E35-R57 which is present in the AutoDock structure was observed to be broken and replaced by the intermolecular hydrogen bond between R57 and the carboxyl group of the ligand. This swapping of hydrogen bonds was estimated to contribute about -2.5 kcal/mol to binding free energy.

We are encouraged by the ability to distinguish true binders from false positives using free energy simulations, as well as the dynamic and physical insights they provided. The current results for the flap allosteric site of HIV Protease demonstrate that the combination of AutoDock docking with BEDAM/DDM could be a powerful tool for ligand screening against promising drug targets.

References:

1. Small Molecule Regulation of Protein Conformation by Binding in the Flap of HIV Protease, T. Tiefenbrunn, et al., ACS Chem. Biol., DOI: 10.1021/cb300611p
2. Binding Energy Distribution Analysis Method (BEDAM) for Estimation of Protein−Ligand Binding Affinities, E. Gallicchio*, M. Lapelosa , and R. M. Levy, J. Chem. Theory Comput., 2010, 6, 2961–2977.

80. Evaluating the Role of Macrocycles in the Susceptibility of Hepatitis C Virus NS3/4A Protease Inhibitors to Drug Resistance

Akbar Ali,[1] Cihan Aydin,[1] Reinhold Gildemeister,[1] Keith P. Romano, Hong Cao,[1] Ayşegül Özen,[1] Djade Soumana,[1] Alicia Newton,[2] Christos J. Petropoulos,[2] Wei Huang,[2] and Celia A. Schiffer[1]

[1]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01605, United States; [2]Monogram Biosciences, South San Francisco, California, United States

The hepatitis C virus (HCV) infects an estimated 150 million people worldwide and is the major cause of viral hepatitis, cirrhosis and liver cancer. The available antiviral therapies, which include PEGylated-interferon, ribavirin and one of the HCV NS3/4A protease inhibitors telaprevir or boceprevir, are ineffective for some patients and cause severe side effects. More potent NS3/4A protease inhibitors are in clinical development, but the long-term effectiveness of these drugs is challenged by the development of drug resistance. Here, we investigated the role of macrocycles in the susceptibility of NS3/4A protease inhibitors to drug resistance in asunaprevir, danoprevir, vaniprevir, and MK-5172, with similar core structures but varied P2 moieties and macrocyclizations. Linear and macrocyclic analogues of these drugs were designed, synthesized and tested against wild-type and drug-resistant variants R155K, V36M/R155K, A156T, and D168A in enzymatic and antiviral assays. Macrocyclic inhibitors were generally more potent, but the location of the macrocycle was critical for retaining activity against drug-resistant variants: the P1–P3 macrocyclic inhibitors were less susceptible to drug resistance than the linear and P2–P4 macrocyclic analogues. In addition, the heterocyclic moiety at P2 largely determined the inhibitor resistance profile, susceptibility to drug resistance, and the extent of modulation by the helicase domain. Our findings suggest that to design robust inhibitors that retain potency to drug resistant NS3/4A protease variants, inhibitors should combine P1–P3 macrocycles with flexible P2 moieties that optimally contact with the invariable catalytic triad of this enzyme.

81. Probing Molecular Interactions of Protease with Small Molecules and Gag Cleavage Junctions, and Matrix with the Lipid Bilayer

Tiefenbrunn, T[1]., Forli, S.[1], Gonzalez, A[2]., Baksh, M. M.[3], Chang, M. W.[4], Tsai, Y.-S.[2], Lin, Y.-C.[5], Perryman, A. L.[1], Rhee, J.-K.[3], Torbett, B.E.[4], Olson, A. J.[1], Soltis, M.[2], Elder, J. H.[5], Finn, M. G.[3], Stout, C. D.[1]

Departments Of [1]ICSB, [3]Chemistry, [4]MEM, [5]IMS, TSRI, 10550 N. Torrey Pines Rd., La Jolla, CA 92037; [2]SSRL, SLAC, 2575 Sand Hill Road MS 99, Menlo Park, CA 95124

A crystallographic fragment-based screen against PR identified two surface sites, the flap site and the exosite, that are potentially important for protein-protein interactions with Gag and are alternatives to the active site for drug design (1). Cocrystals of indole-6-carboxylic acid and 3-indolepropionic acid binding in the flap site were obtained with apo PR with closed flaps (2). A larger BSI phase change was observed for binding to apo PR vs. pepstatin:PR, indicating a larger conformational change upon compound binding in solution, suggesting that compound binding forces the flaps closed.

To find additional hits, a brominated fragment library was screened against a novel $I4_122$ form of W6A PR. Of a library of 68 compounds, one hit was identified in the flap site and one in the exosite. An additional 9 bromine binding sites were found in each site with a strong Br anomalous peak but weak compound density. Br sites provide insight into the nature of the binding pockets, and, for the flap site, show a clear direction for future fragment growth. In the exosite, the movement of Lys14 upon compound binding has motivated computational studies utilizing this different conformation of the exosite as a binding pocket.

Based on prior fragment hits, computational studies in the Olson group identified 1-(4-methylphenyl)sulfonyl-3-(1,3-thiazol-2-yl)urea (C6) as a potential exosite binder. C6 binds to PR as indicated by DSF and BSI assays with 6.5 ºC stabilization and 0.96 +/- 0.11 µM affinity, respectively. Finally, C6 has been co crystallized in two crystal forms of TL-3:PR, conserving interactions observed for other fragments.

In order to visualize detailed, specific interactions of PR with its cleavage junctions in the context of Gag, simplified constructs containing a single cleavage junction, a previously-crystallized Gag domain, and a His tag to ease purification have been designed. The initial constructs include the MA-CA cleavage junction with the NTD of CA followed by His6 and a His tag followed by the CTD of CA and the CA-p2 cleavage junction. After expression, these proteins will be set up in co crystallization trials with D25A, which we have found to be more stable than D25N. Such Gag component complexes address PR binding to a cleavage site while potentially interacting with an adjacent Gag domain via protein–protein contacts. In general, this approach affords an opportunity to address key structural aspects of PR – Gag interaction by using complexes of smaller polypeptides more amenable to crystallization.

Finally, crystal structure of MA in the context of a bilayer is being pursued using the lipidic cubic phase (LCP) method. A MA construct is fused at its N-terminus with a β-barrel known to crystallize in the LCP, replacing the myristoyl chain. Initially, the gene for the *Y. pestis* Ail protein (3QRA) fused at its C-terminus with MA has been synthesized, expressed as inclusion bodies in *E. coli*, and purified in high yield via an N-terminal His-tag on Ail. Protein folding will be assessed via gel shift assay (for the β-barrel Ail protein), size exclusion chromatography, and SAXS prior to the initiation of crystallization trials.

List of Participants

Jinwoo Ahn
University of Pittsburgh School of Medicine
Email: jia12@pitt.edu

Chris Aiken
Vanderbilt University
Email: chris.aiken@vanderbilt.edu

Hashim Al-Hashimi
University of Michigan
Email: hashimi@umich.edu

Tom Alber
UC Berkeley
Email: tom@ucxray.berkeley.edu

Ayna Alfadhli
Oregon Health and Science University
Email: alfadhli@ohsu.edu

Akbar Ali
UMass Medical School
Email: akbar.ali@umassmed.edu

William Allen
Stony Brook University
Email: william.joseph.allen@gmail.com

Luigi Alvarado
University of Maryland, College Park
Email: lja@umd.edu

Zandrea Ambrose
University of Pittsburgh School of Medicine
Email: zaa4@pitt.edu

Brett Anderson
University of Minnesota - Twin Cities
Email: ande8365@umn.edu

Mark Andrake
Fox Chase Cancer Center
Email: mark.andrake@fccc.edu

Rachna Aneja
Drexel University college of medicine
Email: aneja.rachna@gmail.com

Eddy Arnold
CABM + Rutgers University
Email: arnold@cabm.rutgers.edu

Yun Bai
University of California, Berkeley
Email: yunbai@berkeley.edu

Lauren Bailey
Lauren D. Bailey
Email: laurendbailey@gmail.com

Koyeli Banerjee
Michigan State University
Email: koyeli@chemistry.msu.edu

Mary Barkley
Case Western Reserve University
Email: mdb4@case.edu

Joseph Bauman
Rutgers University
Email: bauman@cabm.rutgers.edu

Karen Beemon
Johns Hopkins Univ.
Email: klb@jhu.edu

Richard Belew
UC San Diego
Email: rbelew@ucsd.edu

Carole Bewley
NIH
Email: caroleb@mail.nih.gov

Jayendra Bhonsle
Astha Drug Discovery & Research
Email: jbhonsle@asthaddr.com

Paul Bieniasz
Aaron Diamond AIDS Research Center
Email: pbienias@adarc.org

Pamela Bjorkman
California Institute of Technology
Email: bjorkman@caltech.edu

Marina Bleck
The Rockefeller University
Email: fixm@rockefeller.edu

Ioana Boeras
The Ohio State University
Email: boeras.1@osu.edu

Markus-Frederik Bohn
UMass Medical School
Email: markusf.bohn@umassmed.edu

Ravi Shankar Bojja
ICR,Fox Chase Cancer Center-Temple Health
Email: ravishankar.bojja@fccc.edu

Rosalina Bray
NIHNICHD
Email: brayro@mail.nih.gov

Melanie Brewer
UCSF
Email: melanie.brewer@ucsf.edu

John Briggs
EMBL
Email: briggs@embl.de

Teresa Brosenitsch
University of Pittsburgh
Email: tab24@pitt.edu

Anissa Brown
NIH Office of AIDS Research
Email: brownani@mail.nih.gov

Jonathan Brown
ProSpect Pharma
Email: jmbrown@prospectpharma.com

Lola Brown
UMBC
Email: lolabrown.umbc@gmail.com

Sam Butcher
UW-Madison
Email: sebutcher@wisc.edu

In-Ja Byeon
Universyity of Pittsburgh
Email: ilb6@pitt.edu

Chang Byeon
University of Pittsburgh
Email: chb49@pitt.edu

Yiwei Cao
Stony Brook University
Email: emotianshicyw@gmail.com

Lars-Anders Carlson
NIH
Email: carlsonl@mail.nih.gov

Stacy Carrington-Lawrence
NIH OD
Email: carringtons@od.nih.gov

David Case
Rutgers University
Email: case@biomaps.rutgers.edu

Luis Castillo
Dana Farber Cancer Institute
Email:
luisr_castillomenendez@dfci.harvard.edu

Irwin Chaiken
Drexel University College of Medicine
Email: ichaiken@drexelmed.edu

Max Chang
The Scripps Research Institute
Email: mchang@scripps.edu

Jianbo Chen
NCI-Frederick
Email: chenjia@mail.nih.gov

Yifan Cheng
University of California San Francisco
Email: ycheng@ucsf.edu

Wei Cheng
University of Michigan
Email: chengwe@umich.edu

Peter Cherepanov
London Research Institute
Email: peter.cherepanov@cancer.org.uk

Jean Chin
NIGMS-NIH-DHHS
Email: chinj@nigms.nih.gov

Wah Chiu
Baylor College of Medicine
Email: wah@bcm.edu

Joel Courter
University of Pennsylvania
Email: jcourter@sas.upenn.edu

Charles Craik
UCSF
Email: charles.craik@ucsf.edu

Jonathan D'Gama
Harvard University
Email: jdgama@college.harvard.edu

Victoria D'Souza
Harvard University
Email: dsouza@mcb.harvard.edu

Kalyan Das
Rutgers University
Email: kalyan@cabm.rutgers.edu

T Kwaku Dayie
University of Maryland
Email: dayie@umd.edu

Asim Debnath
Lindsley F Kimball Research Institute
Email: adebnath@nybloodcenter.org

Maria Delucia
University of Pittsburgh
Email: mld80@pitt.edu

Thorsten Demberg
NCI
Email: dembergt@mail.nih.gov

Nanjie Deng
Rutgers University
Email: nanjie.deng@gmail.com

Belete Desimmie
NCI
Email: belete.desimmie@nih.gov

Jennifer Doudna
University of California, Berkeley HHMI
Email: doudna@berkeley.edu

Michael Durney
Harvard University
Email: mdurney@gmail.com

Julie Ealy
Penn State University Lehigh Valley
Email: jbe10@psu.edu

John Elder
The Scripps Research Institute
Email: jelder@scripps.edu

Alan Engelman
Dana-Farber Cancer Institute
Email: alan_engelman@dfci.harvard.edu

Kelly Fahrbach
Northwestern University
Email: k-fahrbach1@northwestern.edu

Anthony Felts
Rutgers University
Email: felts@lutece.rutgers.edu

Bayard Fetler
One Moon Scientific
Email: bfetler@gmail.com

Diana Finzi
DAIDS
Email: dfinzi@nih.gov

William Flynn
Rutgers, The State University of New Jersey
Email: wflynny@physics.rutgers.edu

Brian Fochtman
Stony Brook University
Email: brianfochtman@gmail.com

Valery Fokin
The Scripps Research Institute
Email: fokin@scripps.edu

Alan Frankel
UCSF
Email: frankel@cgl.ucsf.edu

Krista Frankenberry
NCI-Frederick
Email: frankenk@mail.nih.gov

Ernesto Freire
Johns Hopkins Univ.
Email: ef@jhu.edu

Fred Friedman
NIH
Email: fred.friedman@nih.gov

Adam Frost
University of Utah
Email: frost@biochem.utah.edu

James Fuchs
The Ohio State University
Email: fuchs.42@osu.edu

Mariano Garcia-Blanco
Duke University
Email: m.garciablanco@duke.edu

Divita Garg
The Scripps Research Institute
Email: dgarg@scripps.edu

Ritu Gaur
NCI
Email: ritugaur31@gmail.com

Deborah Girma
UMBC
Email: dgirma@gmail.com

Miriam Gochin
Touro University CA
Email: miriam.gochin@tu.edu

Stephen Goff
Columbia University
Email: spg1@columbia.edu

Radoslav Goldman
Georgetown University
Email: rg26@georgetown.edu

David Goodsell
The Scripps Research Institute
Email: goodsell@scripps.edu

J. Rafael Gorospe
NIHNIGMS
Email: gorospejr@mail.nih.gov

David Green
Stony Brook University
Email: david.green@stonybrook.edu

Patrick Griffin
The Scripps Research Institute
Email: pgriffin@scripps.edu

John Grime
University of Chicago
Email: jgrime@uchicago.edu

Angela Gronenborn
University of Pittsburgh
Email: amg100@pitt.edu

John Gross
UCSF
Email: jdgross@cgl.ucsf.edu

David Grunwald
UMass Medical School
Email: david.grunwald@umassmed.edu

Mohan Kumar Haleyur Giri Setty
FDACBER
Email: mohan.haleyurgirisetty@fda.hhs.gov

J Hammond
The Scripps Research Institute
Email: hammondj@scripps.edu

Yun Han
University of Pittsburgh
Email: yuh52@pitt.edu

Han Han
University of Utah
Email: han.han@biochem.utah.edu

Daniel Harki
University of Minnesota
Email: daharki@umn.edu

Reuben Harris
University of Minnesota
Email: rsh@umn.edu

Xiao Heng
HHMIUMBC
Email: hxiao1@umbc.edu

Katrina Heyrana
Penn State College of Medicine
Email: kjh289@psu.edu

Benjamin Himes
University of PIttsburgh School of Medicine
Email: bah80@pitt.edu

William Ho
Rutgers University
Email: who@cabm.rutgers.edu

Andrew Holmes
Drexel University College of Medicine
Email: aph52@drexel.edu

Katie Howell
University of Maryland
Email: khowell06@gmail.com

Wei-Shau Hu
NCI
Email: wei-shau.hu@nih.gov

Stephen Hughes
National Cancer Institute
Email: hughesst@mail.nih.gov

Amy Hulme
Northwestern University
Email: a-hulme@northwestern.edu

James Hurley
NIDDK, NIH
Email: james.hurley@nih.gov

Syed Hussan
NIAID
Email: hussans@mail.nih.gov

Rossi Irobalieva
Baylor College of Medicine
Email: irobalie@bcm.edu

Rieko Ishima
U of Pittsburgh
Email: ishima@pitt.edu

Michelle Itano
The Rockefeller University
Email: mitano@rockefeller.edu

Janet Iwasa
University of Utah
Email: jiwasa@gmail.com

Taisuke Izumi
National Cancer Institute
Email: izumit@mail.nih.gov

Katija Jelicic
NIH
Email: jelicick@niaid.nih.gov

Lingling Jiang
Stony Brook University
Email: jianglilian68@gmail.com

Bruce Johnson
One Moon Scientific, Inc.
Email: bruce@onemoonscientific.com

Barry Johnson
NCI-Frederick
Email: barryj2@mail.nih.gov

Marc Johnson
University of Missouri
Email: marcjohnson@missouri.edu

Christopher Jones
NIH
Email: jones.cprice@gmail.com

Kellie Jurado
Harvard University & Dana Farber Cancer Institute
Email: kelliejurado@fas.harvard.edu

Rebecca Kaddis
Penn State College of Medicine
Email: rjk297@psu.edu

Jason Kaelber
Baylor College of Medicine
Email: kaelber@bcm.edu

Ioannis Kagiampakis
NCI Frederick
Email: kagiampakisin@mail.nih.gov

R Venkat Kalyana Sundaram
Drexel University College of Medicine
Email: vki22@drexel.edu

Virginia Kan
VA Medical Center
Email: virginia.kan@va.gov

Sarah Keane
HHMI,UMBC
Email: keanes@umbc.edu

Vineet Kewalramani
National Cancer Institute
Email: vineet@mail.nih.gov

Tulasi Khandan
Penn State College of Medicine
Email: tkhandan@hmc.psu.edu

Gabriella Kiss
Emory University
Email: gkiss@emory.edu

Madhavi Kolli
UMass Medical School
Email: madhavi.kolli@umassmed.edu

Nicole Koropatkin
Univ of MI Medical School
Email: nkoropat@umich.edu

Moshe Kotler
The Hebrew University
Email: moshek@ekmd.huji.ac.il

Nevan Krogan
University of California, San Francisco
Email: nevan.krogan@ucsf.edu

Troy Krzysiak
PCHPI
Email: trk8@pitt.edu

Lillian Kuo
NCATS
Email: kuols@mail.nih.gov

Nese Kurt-Yilmaz
UMASS Medical School
Email: nese.kurtyilmaz@umassmed.edu

Mamuka Kvaratskhelia
The Ohio State University
Email: kvaratskhelia.1@osu.edu

Peter Kwong
Vaccine Research Center, NIAIDNH
Email: pdkwong@nih.gov

Sook Kyung-Lee
The University of North Carolina at Chapel Hill
Email: sookkyung_lee@med.unc.edu

Judith Lalonde
Bryn Mawr College
Email: jlalonde@brynmawr.edu

Rajan Lamichhane
The Scripps Research Institute
Email: rajan@scripps.edu

Su-Lin Lee
NIDDK
Email: lees24@mail.nih.gov

Kyeongeun Lee
NCINIH
Email: leekye@mail.nih.gov

Lisa Lee
Christian Community Community Health Center
Email: lisa.lee@cchc-rchm.org

Jeffrey Levengood
Case Western Reserve University
Email: jdl108@case.edu

Judith Levin
NIH
Email: levinju@mail.nih.gov

Ronald Levy
Rutgers University
Email: ronlevy@lutece.rutgers.edu

Catherine Lewis
NIGMS, NIH
Email: lewisc@nigms.nih.gov

Ming Li
University of Minnesota
Email: mingli@umn.edu

Wenjun Li
NCBI
Email: wenjun2006@gmail.com

Xing Li
Dana-Farber Cancer Institute
Email: xing_li@dfci.harvard.edu

Chuang Liu
University of Pittsburgh
Email: chl165@pitt.edu

Bindong Liu
Meharry Medical College
Email: bliu@mmc.edu

Sandra Loesgen
NIH
Email: sandra.loesgen@nih.gov

Katheryn Lohith
NIDDK
Email: lohithkm2@mail.nih.gov

Andrew Longhini
UMD-CP
Email: andrew.longhini@gmail.com

Manman Lu
University of Delaware
Email: lumm@udel.edu

Jeremy Luban
UMASS Medical School
Email: jeremy.luban@umassmed.edu

Yi Luo
MEHARRY MEDICAL COLLEGE
Email: yluo@mmc.edu

Sabrina Lusvarghi
NIDDK
Email: sabrinal@mail.nih.gov

Yuri Lyubchenko
UNIVERSITY OF NEBRASKA MEDICAL CENTER
Email: ylyubchenko@unmc.edu

M. Teresa M. Giret
University of Miami
Email: tgiret@med.miami.edu

Suvrajit Maji
University of Pittsburgh School of Medicine
Email: sum48@pitt.edu

José Maldonado
University of Minnesota
Email: jmaldo@umn.edu

Louis Mansky
University of Minnesota
Email: mansky@umn.edu

Youdong (Jack) Mao
Dana-Farber Cancer Institute
Email: youdong_mao@dfci.harvard.edu

Joseph Marcotrigiano
Rutgers University
Email: jmarco@cabm.rutgers.edu

Garland Marshall
Washington University
Email: garlandm@gmail.com

Sergio Martinez
Rutgers, the State University of NJ
Email: martinez@cabm.rutgers.edu

Hiroshi Matsuo
University of Minnesota
Email: matsu029@umn.edu

Angela Mckoy
Rutgers University
Email: afmckoy@gmail.com

Jennifer Mehrens
University of Pittsburgh
Email: jem203@pitt.edu

David Millar
The Scripps Research Institute
Email: millar@scripps.edu

Roger Miller
NIAID
Email: rmiller@niaid.nih.gov

Mithun Mitra
NIH
Email: mitramit@mail.nih.gov

Walther Mothes
Yale University
Email: walther.mothes@yale.edu

Joachim Mueller
University of Minnesota
Email: mueller@physics.umn.edu

James Munro
Yale University
Email: james.munro@yale.edu

Smita Nair
National Cancer Institute
Email: nairs2@mail.nih.gov

Fatima Nawaz
NIAID
Email: nawazf@niaid.nih.gov

Mary Nguyen
OAR, NIH
Email: nguyenmary@od.nih.gov

Olga Nikolaitchik
NCI-Frederick
Email: nikolaio@mail.nih.gov

Jiying Ning
University of Pittsburgh
Email: jin7@pitt.edu

TJ Nitz
DFH Pharma, Inc.
Email: tjnitz@comcast.net

Mariia Novikova
NCI
Email: maria.novikova@nih.gov

Ina O'Carroll
NIH-NCI
Email: ocarrolli@mail.nih.gov

Arthur Olson
The Scripps Research Institute
Email: olson@scripps.edu

Ilker Oztop
Harvard University
Email: ioztop@fas.harvard.edu

Eduardo Paredes
NIH
Email: eduardo.paredes@nih.gov

Leslie Parent
Penn State College of Medicine
Email: lparent@psu.edu

Disha Patel
Rutgers University
Email: dpatel@cabm.rutgers.edu

Vinay Pathak
NCI
Email: pathakv@mail.nih.gov

Juan Perilla
University of Illinois Urbana-Champaign
Email: juan@ks.uiuc.edu

John Perry
The Scripps Research Institute
Email: jjperry@scripps.edu

Alex Perryman
The Scripps Research Institute
Email: perryman@scripps.edu

Boris Peterlin
UCSF
Email: matija.peterlin@ucsf.edu

Eva Petrakova
NIAID - NIH
Email: petrakoe@mail.nih.gov

Tatyana Polenova
University of Delaware
Email: tpolenov@udel.edu

Barbie Pornillos
University of Virginia
Email: bpornillos@virginia.edu

Klara Post
NICHD
Email: postk@mail.nih.gov

Marc Potempa
The University of North Carolina at Chapel Hill
Email: potempa@email.unc.edu

Kristina Prachanronarong
University of Massachusetts Medical School
Email:
kristina.prachanronarong@umassmed.edu

Shiv Prasad
National Institutes of Health
Email: prasads@csr.nih.gov

Peter Preusch
Natl Inst Gen Med Sci
Email: preuschp@nigms.nih.gov

Peter Prevelige
UAB
Email: prevelig@uab.edu

Tuoling Qiu
Stony Brook University
Email: qiutuoling8888@gmail.com

Debra Ragland
University of Massachusetts Medical School
Email: debra.ragland@umassmed.edu

Vijayan Ramaswamy
Rutgers University
Email: suyambu.vijayan@rutgers.edu

Jonathan Rawson
University of Minnesota
Email: rawso018@umn.edu

Elzabeth Read-Connole
NCI Div of Cancer Biology
Email: bconnole@mail.nih.gov

Mike Reddy
NIGMS
Email: michael.reddy@nih.gov

Terry-Elinor Reid
Howard University
Email: yasmanii@hotmail.com

Alan Rein
NCI
Email: reina@mail.nih.gov

David Rekosh
University of Virginia
Email: dr4u@virginia.edu

Andrew Rice
Baylor College of Medicine
Email: arice@bcm.edu

Isis Rivera-Walsh
PSU Hershey College of Medicine
Email: idr100@psu.edu

Robert Rizzo
Stony Brook University
Email: rizzorc@gmail.com

Isaac Rodriguez-Chavez
NIH, NIDCR
Email: isaac@nidcr.nih.gov

Arangassery Rosemary Bastian
Drexel University
Email: rab65@drexel.edu

Michael Rossmann
Purdue University
Email: mr@purdue.edu

Ammeret Rossouw
UMass Medical School
Email: ammeret.rossouw@umassmed.edu

Jamil Saad
University of Alabama at Birmingham
Email: saad@uab.edu

Michael Sakalian
NIGMS
Email: sakalianm@nigms.nih.gov

Carolina Salguero
Harvard University
Email: csalguero@fas.harvard.edu

Daniel Santiago
The Scripps Research Institute
Email: dsantiag@scripps.edu

Stefan Sarafianos
University of Missouri
Email: sarafianoss@missouri.edu

Vern Schramm
Albert Einstein College of Medicine
Email: vern.schramm@einstein.yu.edu

Ursula Schulze-Gahmen
UC Berkeley
Email: uschulze-gahmen@lbl.gov

Jennifer Seedorff
NIHNIAID
Email: jennifer.seedorff@nih.gov

Shivender Shandilya
Univ. Mass. Medical School
Email:
shivender.shandilya@umassmed.edu

Opendra Sharma
DAIDS, NIAID
Email: osharma@niaid.nih.gov

Tania Silvas
UMass Medical
Email: tvsilvas@yahoo.com

Sanford Simon
Rockefeller University
Email: simon@rockefeller.edu

Dustin Singer
NICHD
Email: singerde3@mail.nih.gov

Parmit Singh
NICHD
Email: singhpk@mail.nih.gov

Anna Marie Skalka
Fox Chase Cancer Center
Email: am_skalka@fccc.edu

Marion Skalweit
Cleveland VAMC and CWRU SOM
Email: msh5@case.edu

Jacek Skowronski
Case School of Medicine
Email: jacek.skowronski@case.edu

Janet Smith
University of Michigan
Email: janetsmith@umich.edu

Ferri Soheilian
FNLRF
Email: soheilianf@mail.nih.gov

Mohan Somasundaran
UNIVERSITY OF MASSACHUSETTS
MEDICAL SCHOOL
Email:
mohan.somasundaran@umassmed.edu

Alasdair Steven
NIAMS
Email: stevena@mail.nih.gov

Joshua Strauss
Emory University School of Medicine
Email: joshua.d.strauss@emory.edu

Klaus Strebel
NIAID
Email: kstrebel@nih.gov

Robert Stroud
UCSF
Email: stroud@msg.ucsf.edu

Sriram Subramaniam
National Cancer Institute
Email: ss1@nih.gov

Christopher Suiter
University of Delaware
Email: csuiter@udel.edu

Michael Summers
UMBC
Email: summers@hhmi.umbc.edu

Wes Sundquist
University of Utah
Email: wes@biochem.utah.edu

Ronald Swanstrom
The University of North Carolina at Chapel Hill
Email: risunc@med.unc.edu

Philip Tedbury
NCI
Email: philip.tedbury@nih.gov

Alice Telesnitsky
University of Michigan
Email: ateles@umich.edu

Theresa Tiefenbrunn
TSRI
Email: theresat@scripps.edu

Blanton Tolbert
Case Western Reserve
Email: bst18@case.edu

Bruce Torbett
The Scripps Research Institute
Email: betorbet@scripps.edu

Thao Tran
University of Maryland, Baltimore County
Email: lphthaotran@umbc.edu

Stephanie Tristram-Nagle
Carnegie Mellon University
Email: stn@cmu.edu

Steve Tuske
CABMRutgers
Email: tuske@cabm.rutgers.edu

Emiko Urano
NCI
Email: uranoe@mail.nih.gov

Rachel Van Duyne
NCI-Frederick
Email: rachel.vanduyne@nih.gov

Nancy Vazquez
NIH
Email: nvazquez@niaid.nih.gov

Gregory Voth
University of Chicago
Email: gavoth@uchicago.edu

Joseph Walsh
Touro University. University of California, San Fr
Email: joseph.walsh@tu.edu

Chenliang Wang
Meharry Medical College
Email: chenliangwang1984@gmail.com

Carl Wild
DFH Pharma, Inc.
Email: cwild@aol.com

James Williamson
The Scripps Research Institute
Email: jrwill@scripps.edu

Ian Wilson
The Scripps Research Institute
Email: wilson@scripps.edu

Sandra Wolin
Yale School of Medicine
Email: sandra.wolin@yale.edu

Cora Woodward
California Institute of Technology
Email: cora@caltech.edu

Elizabeth Wright
Emory University
Email: erwrigh@emory.edu

Tiyun Wu
NICHDNIH
Email: wutiyun@mail.nih.gov

Ying Wu
PITT
Email: yiw60@pitt.edu

Jie Xia
Howard University
Email: jie.william.xia@gmail.com

Li Xie
Michigan State University
Email: lixie.chem.xmu@gmail.com

Yong Xiong
Yale Univeristy
Email: yong.xiong@yale.edu

Hongzhan Xu
University of Pittsburgh
Email: xuhongzhan@hotmail.com

Guangai Xue
NCI
Email: xueg@mail.nih.gov

Junpeng Yan
CASE WESTERN RESERVE UNIVERSITY
Email: jxy104@case.edu

Dong Yang
University of Maryland
Email: dyang@umd.edu

Mark Yeager
University of Virginia School of Medicine
Email: yeager@virginia.edu

Ravikiran Yedidi
National Cancer Institute
Email: yedidirs@mail.nih.gov

Zehra Yildiz
Harvard university
Email: fyildiz@fas.harvard.edu

Shujun Yuan
UCSF
Email: yuan@msg.ucsf.edu

Peijun Zhang
University of Pittsburgh
Email: pez7@pitt.edu

Gongpu Zhao
University of pittsburgh
Email: goz1@pitt.edu

Li Zhou
The Scripps Research Institute
Email: lizhou@scripps.edu

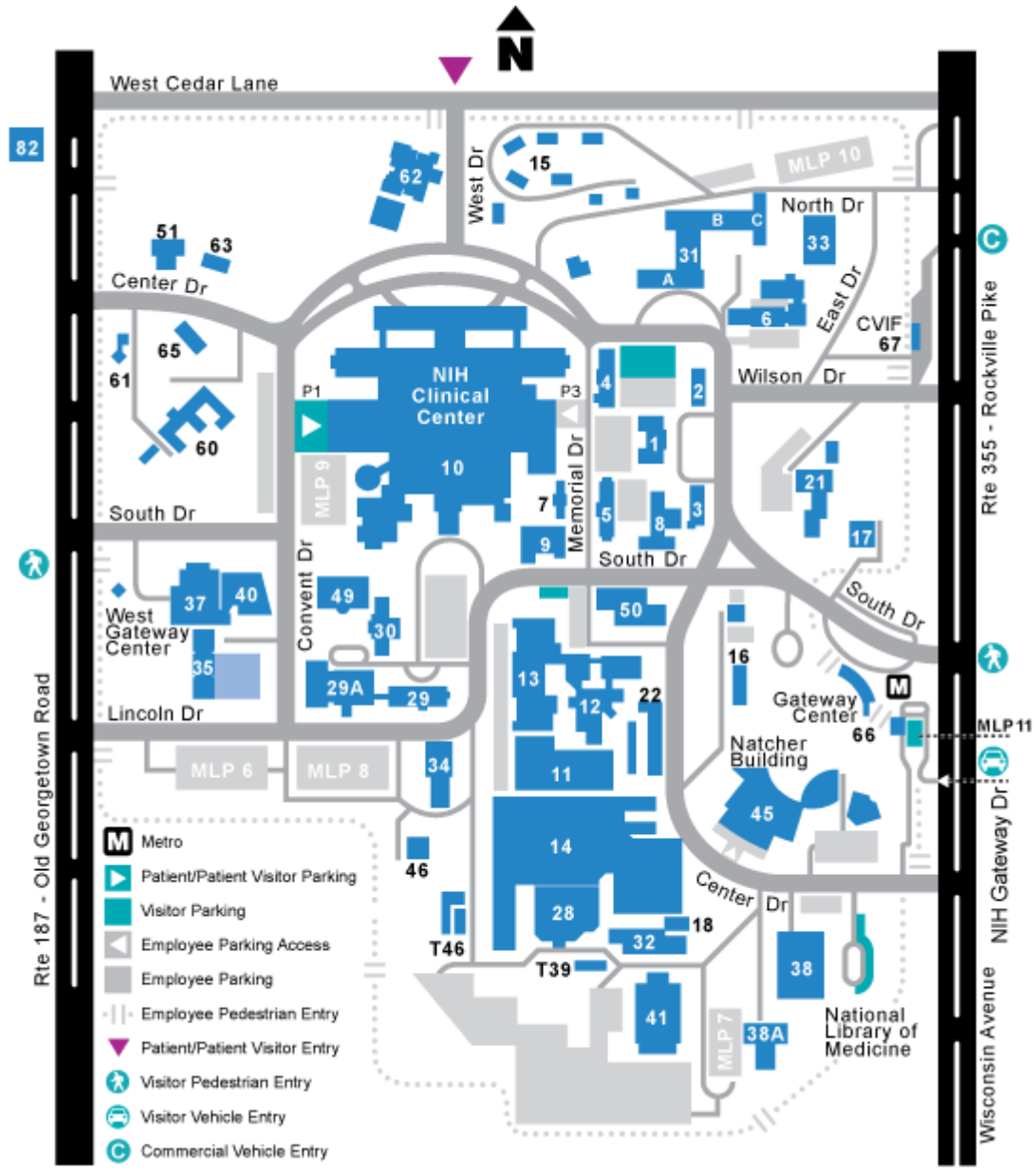
Xiaohong Zhou
Johns Hopkins University
Email: xiaohong.zhou2006@gmail.com

Qiang Zhou
University of California, Berkeley
Email: qzhou@berkeley.edu

Yuchen Zhou
Stony Brook University
Email: chris.yuchen.zhou@gmail.com

BACK

Map of NIH Campus



Some useful notes:

- The conference is located in Natcher (Building 45), southwest of the Gateway Center entrance to campus. The Medical Center Metro stop is next to the Gateway Center.
- Non-NIH employees must undergo inspection and receive temporary ID cards at the Gateway Center (see additional information on following pages).
- Parking garage MLP-11 is for non-NIH employees; NIH employees may park in the garage under Building 45 (requires car safety inspection next to Building 38A) or in other employee parking.
- Dining options on the NIH campus include Eurest Dining Services locations in Buildings 45, 1, 10, and 31, Maryland Business Enterprise Program for the Blind locations in Buildings 38A and 12B, and a concession stand in the Natcher lobby (http://does.ors.od.nih.gov/food/index.htm).
- An ATM is located in the Natcher lobby.

BACK

NIH Gateway Center Map

Main Visitor Entrance: NIH Gateway Drive
Gateway Center - Building 66 (for pedestrians entering campus)

Gateway Inspection Station - Building 66A (for vehicles entering campus)

• Monday – Friday: 5am – 10pm; Weekends and After Hours: Closed After hours: After 10pm on weekdays, all day weekends and holidays, pedestrians and visitors in vehicles should enter campus via the Commercial Vehicle Inspection Facility (CVIF) - Building 67 (on Rockville Pike between North Drive and Wilson Drive)
• After inspection, vehicles enter campus at Center Drive
• Roadway at Center Drive is for entering campus only; visitors exiting campus may exit from other open locations. To see a list of exits, please see the map.
• All vehicles and their contents will be inspected upon entering the campus.

Multi-Level Parking Garage 11 – MLP-11 (for parking outside of campus)

• Monday – Friday: 6am – 9pm (entrance) 6am – 11pm (exit) Weekends: Closed
• When MLP-11 is closed, visitors can park in lots on the NIH Campus
• Visitors parking in this garage should proceed to the Gateway Center (Bldg. 66) to get a visitor badge
• All visitors traveling in a vehicle are highly encouraged to park in MLP-11 as there is limited visitor parking on the main campus
• No vehicle inspection required to park in MLP-11
• Vehicles left in the MLP-11 parking garage after 11pm on weekdays or during any weekends are subject to ticketing and towing
• Cost: $2 per hour for the first three hours, $12 maximum for the entire day

Directions to NIH Gateway Drive from Rockville Pike/Wisconsin Avenue:
Southbound:

1. Continue on Rockville Pike past South Drive
2. Turn right at NIH Gateway Drive

Northbound – Option 1:

1. Continue on Rockville Pike past South Drive
2. Make a u-turn from the left turn lane at Wilson Drive
3. Continue southbound on Rockville Pike past South Drive
4. Turn right at NIH Gateway Drive

Northbound – Option 2:

1. Continue on Rockville Pike
2. Turn left at Battery Lane
3. Turn right on Old Georgetown Road
4. Turn right on Cedar Lane
5. Turn right on Rockville Pike
6. Continue southbound on Rockville Pike past South Drive
7. Turn right at NIH Gateway Drive

Northbound – Option 3:

1. Continue on Rockville Pike to South Drive
2. Make a u-turn from the left turn lane at South Drive
3. Continue southbound on Rockville Pike
4. Turn right at NIH Gateway Drive

Security Procedures for Entering the NIH Campus:
* All visitors and patients—**please be aware**: Federal law prohibits the following items on Federal property: firearms, explosives, archery equipment, dangerous weapons, knives with blades over 2 ½ inches, alcoholic beverages and open containers of alcohol.
* The NIH has implemented security measures to help ensure the safety of our patients, employees, guests and facilities. All visitors must enter through the NIH Gateway Center at Metro or the West Gateway Center. You will be asked to submit to a vehicle or personal inspection.
* Visitors over 15 years of age must provide a form of government-issued ID such as a driver's license or passport. Visitors under 16 years of age must be accompanied by an adult.

Vehicle Inspections – All vehicles and their contents will be inspected upon entering the campus. Additionally, all vehicles entering certain parking areas will be inspected, regardless of any prior inspection. Drivers will be required to present their driver's license and may be asked to open the trunk and hood. If you are physically unable to perform this function, please inform the inspector and they will assist you.
Vehicle inspection may consist of any combination of the following: Detection Dogs Teams (K-9), Electronic Detection Devices and Manual Inspection.
After inspection, you will be issued a vehicle inspection pass. It must be displayed on your vehicle's dashboard while you are on campus. The inspection pass is not a "parking permit." It only grants your vehicle access to enter the campus. You can only park in designated parking areas.

Personal Inspections – All visitors should be prepared to submit to a personal inspection prior to entering the campus. These inspections may be conducted with a handheld monitoring device, a metal detector and by visible inspection. Additionally, your personal belongings may be inspected and passed through an x-ray machine.

Visitor passes must be prominently displayed at all times while on the NIH campus.
To learn more about visitor and security issues at the NIH, visit:
http://www.nih.gov/about/visitor/index.htm.
For questions about campus access, please contact the ORS Information Line at orsinfo@mail.nih.gov or 301-594-6677, TTY - 301-435-1908.